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FLORA W. PATTERSON

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Mrs. Flora W. Patterson, for more than 25 years a member of the staff of the Bureau of Plant Industry, United States Department of Agriculture, died February 5, 1928.

Those of us who measure service in the field of mycology in decades rather than years can look back the somewhat misty pathway and recall our struggles to orient ourselves when mycology began to be pushed into the background by the urgent demand on the part of the public for help in meeting the ravages of numerous crop diseases. There was a period in the early 80's when a mycologist might go his peaceful way collecting and delving in species to his heart's content without fear or distractions of any kind. Mrs. Patterson came to the Department just about the close of this period. The mycologist was passing. The glamor of field service in phytopathology, with its attendant large contacts and large problems, was irresistible so that our collections and herbaria were beginning to languish and our mycological technique becoming rusty for lack of use. To meet the situation, insofar as it related to the Department's work, we tried various expedients and made numerous experiments. It was the conviction of my colleagues of the period that our only hope was to find a good man, rich in experience and so wedded to mycology and its attendant interests that nothing could swerve him from the then recognized beaten path. The experiment was made, but the man failed us.

In our search for relief, we became acquainted with Mrs. Patterson and her work, and on January 15, 1895, she came to us as Assistant Pathologist. Special note should be made of the title, as it is doubtful if we could have succeeded in having her appointed as a mycologist. It soon became evident that Mrs. Patterson's heart and soul were in the work of making our collections something really worth while. This was six or seven years before the organization of the Bureau of Plant Industry and during a period when the groundwork was being laid for the expansion of many lines of botanical work in the Department. In June, 1901, just before the law creating the Bureau of Plant Industry became effective, Mrs. Patterson was made Mycologist, which title she held through more than twenty years of further service in the Department.

It was my privilege and pleasure to be in rather close touch with Mrs. Patterson's work during her long period of service ending in April, 1923. In all that time her loyalty to the collections and all they stood for was unfaltering and unswerving. Men and women were coming into the Bureau and moving rapidly forward in the broad field of phytopathology. There was great demand and pressure for workers in this field and the opportunities for advancement were numerous. Mrs. Patterson, so far as I can recall, never became interested in the excitement around her. She had her job and she stuck to it to the end. As the burdens put upon the Bureau became greater, Mrs. Patterson did her share toward lightening them. Thus, when it became necessary to put into operation the Plant Quarantine Law of 1912, Mrs. Patterson and her assistants rendered material aid in organizing and setting in motion the pathological inspection service which has since grown into large proportion.

Mrs. Patterson possessed an active mind and her interests extended much beyond the field of her special work. In the early years of the Bureau of Plant Industry, when policies were being shaped and the groundwork was being laid for an organization in which everyone engaged in research might have freedom of action and the sympathetic support of those engaged in related lines of work, Mrs. Patterson proved a helpful and able adviser. She was cheerful at all times, optimistic in the face of difficulties, and forward looking in all matters pertaining to her work.

Anyone familiar with the steady grind and labor connected with species identification, care of specimens, keeping up with exsiccatae, exchanging plant material, indexing systematic mycological literature, and a multitude of other things associated with a large herbarium in constant use by a score of workers in phytopathology, will realize that little time is left for original research. Despite these handicaps and interruptions, Mrs. Patterson found time to turn out some creditable pieces of research. Her ambition, however, was to serve others by lightening their burdens wherever and whenever possible.

Of the personal side of her life we have little knowledge. We know that she came of good stock, her father being a Methodist minister. We know that for years she had the care of her husband, who, through an accident, was a helpless invalid. Despite these heavy burdens, Mrs. Patterson prepared her two sons for college and managed to enter herself at Radcliffe. She remained at Radcliffe three years, taking special work in botany while working as an assistant in the Gray Herbarium at Harvard. It was while at the Gray Herbarium that she received her special training in mycology and the care of mycological collections. Mrs. Patterson came to the Department direct from the Gray Herbarium, having qualified in 1895 through the usual Civil Service channels. Under the terms of the United

States Retirement Act of 1920, Mrs. Patterson was registered for retirement in 1922. Her period of service, however, was extended for another year, her final retirement taking place April 20, 1923. After retiring, Mrs. Patterson lived quietly with her son. She passed away at the home of her son in Brooklyn, New York. Her friends and co-workers will long remember her as one who could smile through shadow and sunshine, one who was always willing to help, and one whose loyalty to her chosen field remained steadfast to the end.

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PHYTOPHTHORA BLIGHT OF PEONY[1]

D. C. Cooper and C. L. Porter

The fungus which causes peony blight rather closely resembles *Phytophthora cactorum* and *P. fagi* but has been found to differ from them in morphology, cultural characters, and parasitic capabilities, and is herein designated as *Phytophthora paeoniae*, n. sp. The outstanding morphological characteristics of this fungus are its relatively broad conidia with flat papillae, long twisting antheridial stalks, and more or less spherical haustoria. Only young growing tissues of the peony plant are susceptible to infection, and in the stem the mycelium invades most rapidly the pericycle, medullary rays, and pith.

Oogonia and antheridia are produced in great abundance in pure culture and precede the conidia, the production of which is stimulated by lack of nutrient substances.

Spore production ceased under red and yellow-orange lights. A variety of peculiar morphological and physiological responses were made by the fungus when exposed to the vapors of different volatile substances such as acetone, alcohol, turpentine, oil of bergamot, oil of eucalyptus, clove oil, thymol, menthol, CO_2, and illuminating gas.

Thurston and Orton (12) noted a disease of peony near Erie, Pennsylvania, in 1921, caused by a species of *Phytophthora* which they briefly described. In 1922 a similar disease was reported by White (see Martin, 4, p. 439) as occurring at Shawnee, Kansas. At Hampden, Connecticut, Clinton (see Martin, 5, p. 403) observed the disease and made isolations of the fungus. He obtained both a *Botrytis* and a *Phytophthora* from the blighted stems but considered the *Phytophthora* to be the first invader.

At Lafayette, Indiana, in 1924, Gardner (2, p. 256) observed the disease to be rather serious on the more succulent parts of the plant where it occurred as a stem rot and leaf spot. During the early part of May, 1927, Leslie Pierce, of the U. S. Department of Agriculture, collected a number of blighted stalks in a seven-acre peony farm near Vincennes, Indiana, where the disease was especially prevalent on the varieties Felix Crouse and Festiva maxima. Professor H. H. Whetzel, in a letter to the writers, reported that the blight occurred in New York in 1927. On May 1, 1928, the disease was again observed by Pierce near Vincennes, who said that the peony varieties Felix Crouse, Carl Rosenfeld, and Floral Treasure were

[1] Contribution of the Biological Laboratories, Purdue University, Lafayette, Indiana.

very susceptible to the disease and that the varieties Festiva maxima and Edulis superba were moderately susceptible. Diseased plants were also obtained from a peony grower near Battle Ground, Indiana.

It is impossible to make an estimate of the total loss caused by the disease. Since it is especially destructive to the growing tips, it will suffice to say that the Phytophthora blight is of considerable importance to the cut-flower peony grower.

SYMPTOMS

The Phytophthora blight attacks the stems, leaves and buds, the symptoms varying with the age of the plant and the point of attack. When the infection occurs near the tip of a shoot, the blight manifests itself as a necrotic condition of the tip including the leaves and extends for several inches down the stem (Fig. 1, A). The parts affected are dark brown or

FIG. 1.—A. Tip blight caused by the peony *Phytophthora*. B. Early stage of stem infection. C. Later stage just before the collapse of the stalk.

black and somewhat leathery. When the point of infection is farther down the stem, a portion of the stalk several inches in length becomes involved and turns black, and the shoot droops over (Fig. 1, B, C). The central portion of the lesion is black shading toward the edge so that the color of the infected portion gradually merges with the reddish color of the healthy stem. When the young stalks are attacked near the ground line, the entire shoot turns black. Observations by Professor H. S. Jackson indicate that this fungus under field conditions may produce a destructive crown rot.

Under ordinary conditions practically no fungous growth appears on the surface of the diseased portion of the plant. When, however, these parts are placed in a moist chamber or the plants are kept in a saturated atmosphere, an abundant white cottony growth appears on the surface of the invaded parts. Numerous conidia are found on this aerial mycelium. No other spore forms have been found on, or in, the host tissue.

PATHOGENICITY OF THE PEONY FUNGUS

The organism causing the Phytophthora blight of peony was isolated May 5, 1927, from material collected by Pierce. The diseased stems were surface sterilized in $HgCl_2$, one part to 1,000, for two minutes, then rinsed in sterile water. Transverse sections of the stem about 5 mm. long were made with a flamed scalpel and placed on potato-dextrose-agar plates. Within 48 hours the mycelium had grown away from the sections and transfers were made to agar slants. The fungus was easily isolated without contamination. Usually agar blocks containing mycelium were used for inoculation purposes but, when desired, zoospores were readily obtained by placing conidia in water.

The pathogenicity of the fungus thus isolated was proved by inoculating healthy peony plants. Roots of the Edulis superba variety were potted in January, 1928, and grown in the Purdue greenhouse. When the stalks had reached a height of 6 inches, the plants were inoculated by placing pieces of the agar containing mycelium around the uninjured stalks and then placing the plants in moist chambers. After a period of 48 hours the plants were removed from the moist chambers and placed on the greenhouse bench. Six days after inoculation definite lesions had formed at the places of inoculation, and the next day the infected stalks had withered and dropped at the point of the lesion (Fig. 5, A). Checks treated in the same way, except that pieces of agar without the mycelium were used, remained healthy. A second series of plants was similarly inoculated and similar results were obtained. Infection was also readily secured by placing a drop of water containing the zoospores in the axil of the leaf. The fungus was reisolated and compared with the original cultures, with which it was found to agree

in all respects. The controls inoculated with water alone showed no signs of infection. It was found that the disease readily spread to peony plants which were in close proximity to the infected plants when these were watered with a hose.

INOCULATION TESTS WITH OTHER HOSTS

Other plants selected for inoculation with this fungus were bean, tomato, cabbage, sunflower, potato, nasturtium, and broad bean. These were inoculated by placing agar blocks containing the mycelium over a fresh scalpel wound. The inoculated plants were placed in a moist chamber for three days and then removed to the greenhouse bench. No infection occurred on any of these species.

Various roots, fruits, and detached leaves were inoculated to ascertain the host range of this fungus. The specimens to be inoculated were immersed in a weak solution of bichloride of mercury, then rinsed in sterile water and placed in a moist chamber. Inoculations were made by placing a block of agar containing the mycelium on the unbroken surface on one specimen and over an injury made with a flamed scalpel on another specimen. These inoculations were made in duplicate, and a third group of substrata receiving the same treatment but not inoculated was run as a check. All were held at room temperature.

After 10 days in a moist chamber uninjured turnips, parsnips, and carrots showed no infection and only a slight discoloration occurred at the point of inoculation on the surfaces cut with the scalpel. The turnip was blackened below the place of inoculation for a depth of 1–2 mm. and mycelium was found in the blackened tissue.

Ripe tomatoes were attacked readily if the agar block was placed over an injury. The fungus did not gain entrance into the uninjured fruit. Green tomatoes and bell peppers, whether injured or not, were not penetrated by the fungus.

This *Phytophthora* invaded and rotted injured apples but could not enter through the unbroken epidermis. The lesion produced did not have a zonate appearance as does that produced by *P. cactorum*.

Although the fungus was placed on detached living leaves of nasturtium, tomato, *Thalictrum*, hollyhock, soy bean, sunflower, *Nicotiana*, broad bean, and peony, it did not enter the tissue except in the last two mentioned. It caused blackened areas on these leaves and the fungus was easily reisolated from the necrotic spots.

RELATION OF THE FUNGUS TO THE HOST TISSUE

For the purpose of studying the relation of the fungus to the host tissue, portions of the infected peony stalks were fixed in a solution of 6.5 cc.

formaldehyde and 2.5 cc. glacial acetic acid in 100 cc. of 50 per cent alcohol. Permanent mounts were made by the paraffin process. The best stain was found to be Fleming's triple with a very light counterstain in Delafield's haematoxylin. By this method the fungus was very easily differentiated from the host tissue.

A careful study of these slides showed that the fungus advanced from the point of inoculation through the cortex to the pericycle and then encircled the stem through the pericycle in both directions. From the pericycle the fungus passed through the medullary rays to the pith and caused a collapse of the pith cells (Fig. 2, A). Unlike the condition found in the

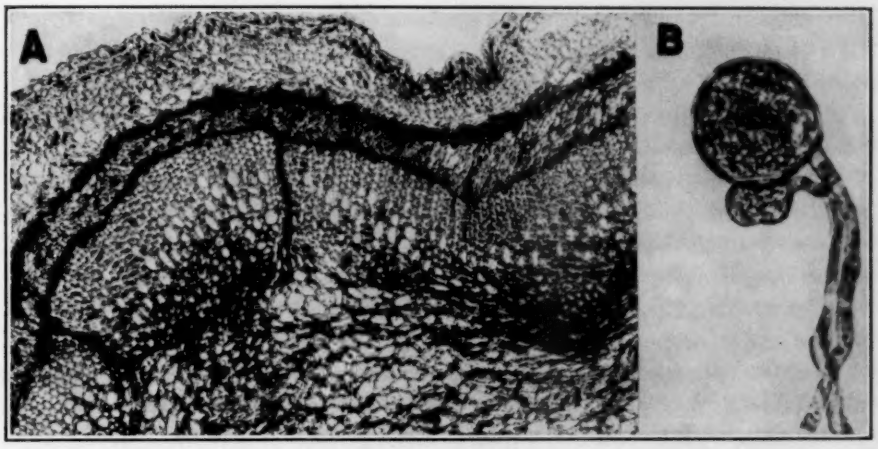

FIG. 2.—A. Cross section of the stem showing pericycle, medullary rays, and pith invaded by the fungus. B. Oogonium and antheridium showing their relatively long stalks and their paragynous relationship.

Phytophthora rots of apple (9), ginseng (10), and strawberry (8), the vascular tissues of the peony were not invaded. The mycelium is intercellular and sends small more or less spherical haustoria into the cells (Plate XXIX, A). The haustoria have nearly the same diameter as the mycelium but are very much constricted at the point where they pass through the cell walls.

CULTURAL CHARACTERS OF THE PEONY FUNGUS

The peony *Phytophthora* has been grown on a number of the ordinary solid culture media, on cooked vegetable plugs, and in liquid media.

On commercial lima bean agar, growth was very abundant, covering a 90 mm. petri plate in 6–7 days. Both aerial and submerged mycelia were produced. The aerial mycelium, being in scattered tufts, gave the culture

a loose cottony appearance. Unlike certain other species of *Phytophthora* the colony was not zonate in appearance, nor did it have radial strands. The formation of oogonia and antheridia preceded that of conidia, the former being produced on mycelium from 24 to 48 hours old and the latter on mycelium from 4 to 6 days old.

On cornmeal agar the aerial mycelium was less abundant than on lima bean agar. The colony increased slowly in diameter, taking from 10 to 15 days to cover a 90-mm. petri plate. Oospores and conidia were produced in the same sequence as on lima bean agar.

On potato dextrose agar the aerial mycelium was less dense than on cornmeal agar. The colony covered a 90-mm. petri plate in 10–12 days. Oospores and conidia were sparingly produced.

On washed agar the growth was very sparse. No oogonia and antheridia were produced. A few conidia were produced on the older mycelium. On a synthetic medium[2] and on Bacto nutrient agar the growth was very slight and no oospores or conidia were produced.

On cooked vegetable plugs, after 30 days, the growth was as follows:

Ripe tomato plugs—heavy mat of mycelium, no conidia, many oospores.

Green tomato plugs—heavy mat of mycelium, few conidia, and occasional oospores.

Carrot, parsnip, sweet potato, and green bean plugs—thick mat of mycelium, many oospores, no conidia.

Pepper plugs—thin growth of mycelium, oospores and conidia in abundance.

Green pea plugs and apple plugs—little growth of mycelium, no oospores or conidia.

Peony stem plugs—thick aerial mycelium, oospores in abundance, no conidia, a few chlamydospores. This is the only medium on which chlamydospores were produced.

When the peony *Phytophthora* was grown submerged in beef bouillon, it produced a dense mycelium with no spores. In soil water the thick growth of mycelium produced conidia but no oospores. When grown in maple sap the mycelium produced oospores but no conidia. In prune decoction the mycelium grew luxuriantly, and when it reached the surface of the liquid produced aerial conidiophores and conidia but no oospores.

MORPHOLOGY OF THE PEONY PHYTOPHTHORA

Mycelium. The hyphae are somewhat geniculate and considerably branched. Septa, as a rule, are lacking except for an occasional one in the

[2] The synthetic agar was made up as follows: distilled water 1,000 cc.; dextrose 100 gms.; peptone 20 gms.; ammonium nitrate 10 gms.; magnesium sulfate (crystals) 2.5 gms.; potassium nitrate 5 gms.; dibasic potassium phosphate 2.5 gms.

older hyphae. The protoplasmic contents are granular and somewhat vacuolate. The hyphae vary from 3 to 10 μ in width. A rapid streaming of the protoplasm in the hyphae may be observed if the culture is examined under the low power of the microscope immediately after the cover of the petri plate is removed. The spore forms will be taken up in the order of their appearance in the colonies grown on lima bean agar.

Sexual organs. For the detailed study of the development of the sexual organs, agar block cultures were used. Slides were prepared by transferring agar blocks from the edge of a petri plate colony to clean slides and placing a cover glass over this agar block. These mounts were kept in moist chambers and examined at intervals.

When permanent mounts were desired, bits of the mycelium were removed from the agar blocks and placed in a drop of 10 per cent formalin for 10 minutes, washed in several changes of water, stained in Congo red and mounted in 10 per cent glycerine. The glycerine was allowed to thicken, a cover glass was put in place, and the mount was sealed with gold size or Duco lacquer. Another method used was to place on a clean slide a drop of lacto-phenol containing 0.5 per cent cotton blue and 0.5 per cent methyl green. In this a bit of the fungus was placed and a cover glass added. With this combination of stains the walls of the oogonium were stained blue and the contents green. These mounts were also made permanent by sealing with gold size or Duco lacquer. When a very thin drop of the mounting medium and a very thin cover glass were used, it was possible to examine these mounts with the oil immersion lens. There was very little distortion of the fungus in either method and the hyphae were beautifully stained.

The oogonia and antheridia arise as terminal swellings of comparatively long, twisting branches from the same or closely adjacent hyphae. (Fig. 1, B; Plate XXIX, G). The antheridium is an ellipsoidal body which is formed somewhat in advance of the thin-walled oogonium. When fully developed the antheridium lies at the base of the oogonium and closely appressed to it. The oogonium when fully developed varies in diameter from 25 to 35 μ. A tube is formed connecting the oogonium and antheridium and through this the contents of the antheridium flow into the oogonium.

The antheridium of this fungus lies at the base of the oogonium, closely appressed to the oogonial stalk. Although occasionally, upon superficial examination, the antheridium appears to have an amphigynous relation to the oogonium such as was described by Murphy (6), yet by careful focusing of the microscope, and by a study of the mode of development of these organs, no case was observed where the oogonium actually grew through

the antheridium (amphigyny) (Plate XXIX, C, D, E). The antheridium is very clearly at the side of the oogonial stalk when observed before fertilization occurs. After the antheridial contents are transferred to the oogonium through the fertilization tube, the antheridium is a flaccid hyaline empty sac which sometimes appears to collapse around the oogonial stalk (Plate XXIX, D).

With the completion of fertilization a thick-walled oospore is formed. These are abundant in older cultures on most agars and vegetable plugs and in soil water. The oospores are globose, with thick walls and a vacuolate central portion, and vary from 24 to 30 μ in diameter, the mode being 26 μ. Oospores from a one-month-old colony germinated by production of a germ tube if they were placed in a weak sugar solution. As previously stated, no oospores have been found either on or in the diseased peony tissue.

Conidia. The conidiophores are formed on the older parts of the colony but have not been found on mycelium less than 4 or 5 days old. They are prostrate on the surface of the medium. Their diameter is slightly less than that of the mycelium from which they arise. Each conidiophore bears from 1 to 15 conidia on short lateral branches, the usual number being from 4 to 8. The conidia are ovoid with broad apical papillae and are pale yellowish gray in color with granular and often vacuolate contents (Plate XXIX, B). They vary in size from 22 to 38 μ by 26 to 42 μ, mostly from 26 to 28 μ by 30 to 33 μ.

Zoospores. The conidia usually germinate by the formation of zoospores. When the conidia are placed in a drop of water the contents surrounding the large central vacuole break up into 20–40 individual units which round up into zoospores (Plate XXIX, B). In a short time these spores exhibit slight movement due to the rapid vibration of the cilia. With the increase in size and activity of the zoospores the central vacuole of the conidium entirely disappears.

The zoospores escape through the apical plug which is ruptured because of the internal movement. Usually this escape is very rapid. Sometimes a few of the zoospores escape and relieve the internal pressure. The remaining spores become very active, each one escaping from the conidium by squeezing through the apical opening or orifice, which has a smaller diameter than the zoospore itself. In the first stage of its escape, the anterior end of the zoospore is squeezed into the orifice. The posterior end then seems to contract, forcing the contents of the spore forward. The protruding anterior end becomes swollen beyond the orifice so that the partially extruded zoospore assumes the shape of an hour-glass. By further contraction the entire contents continue to flow forward, and the spore

aided by its cilia escapes with a spiral movement. In some cases a few of the zoospores do not escape but come to rest and germinate within the conidium (Plate XXIX, B3). When zoospores are not formed within a period of a few hours, the conidium produces germ tubes directly, usually through the apex, sometimes laterally (Plate XXIX, B2).

The zoospores are reniform in shape with two cilia attached near the middle of the concave side. After a short period of brisk activity, the spores gradually come to rest and assume a spherical shape, losing or absorbing the cilia. Almost immediately germ tubes are sent out so that in the space of an hour after the production of zoospores, hyphae are being developed (Plate XXIX, B4).

Chlamydospores. The chlamydospores are spherical and thin-walled with granular contents. They have been observed only on cooked peony stems and then were relatively few in number. The majority of these spores range in diameter from 30 to 40 μ. Germination of the chlamydospores has not been observed.

EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF THE FUNGUS IN CULTURE

Tests were made to determine the optimum temperature for the growth of the fungus. Lima bean agar was used because it was known that the fungus grows best on this medium. Petri plates for each temperature were prepared in duplicate and the entire test repeated to check results. Temperatures of 14° and 18° C. were obtained in a refrigerator and temperatures of 22°, 26°, 30°, 34°, and 37° C. in incubators under thermostatic control. All the cultures in the incubators were kept in moist chambers to prevent the medium from drying. The plates were inoculated and the colonies allowed to grow for one day and then measured before the plates were placed in the constant temperature chambers.

The daily increase in the diameter of the colonies was recorded at 4 p. m. each day for a period of 7 days. The average daily increase at the various temperatures was 3 mm. at 14° C., 7 mm. at 18° C., 14 mm. at 22° and at 26° C., 12 mm. at 30° C., 1 mm. at 34° C., and 0 mm. at 37° C. From these measurements it can be seen that the optimum temperature for the growth of this fungus in culture lies between 22° and 26° C., with a sharp break above 30° C. In every case the fungus was dead when left at 37° C. for a period of 7 days.

Tisdale and Kelley (13) found the optimum temperature for the growth of *Phytophthora nicotianae* to be between 25° and 30° C. Rose (8) considers the best temperature for the growth of *P. cactorum* to be between 25° and 36° C. At room temperature cultures of the peony *Phytophthora*

may be held for periods of two to three months without loss of vitality. Church and Scandiffio (1) also found room temperature best for the storage of Phytophthora cultures.

EFFECT OF THE H-ION CONCENTRATION OF CULTURE MEDIA ON THE GROWTH OF THE FUNGUS

Lima bean agar was made up and, when tested, was found to have a pH of 6.6. This was divided into three parts, one part was used as it was, another was adjusted to a pH of 5.3 by adding lactic acid, and the third part adjusted to a pH of 8.4 by the addition of NaOH. Petri plates of each of the media were prepared in triplicate and after inoculation were held at room temperature for 5 days. An effort was made in all cases to use the same amount of inoculum.

At the end of the test it was found that the colonies on the media having a pH of 5.3 had an average diameter of 32 mm., those on media having a pH of 6.6 had an average diameter of 61 mm. and those on media having a pH of 8.4 had an average diameter of 41 mm. In so far as vegetative growth was concerned, the most favorable H-ion concentration for the fungus was the medium having a pH of 6.6. A microscopic examination revealed the fact that the colonies at a pH of 6.6 and 8.4 bore numerous oospores, whereas the colony growing on agar having a pH of 5.3 bore comparatively few conidia or oospores. Tisdale and Kelley (13), in their work on *Phytophthora nicotianae,* found the H-ion concentration most favorable for vegetative growth of the fungus to be between a pH of 4.4 and pH 5.1.

When brom-cresol purple was added to neutral beef bouillon as an indicator, and the fungus grown submerged in the liquid, there was no color change even though the culture was kept for six weeks, thus indicating no change in the pH.

THE EFFECT OF CERTAIN VARIATIONS OF RICHARD'S SOLUTION ON THE GROWTH OF THE PEONY PHYTOPHTHORA

The peony *Phytophthora* grew well in Richard's solution, producing a thick mat of submerged mycelium but no spores. If the solution was varied by adding either lactose or saccharose for the glucose, there seemed to be no difference in the amount or type of mycelium produced. Even when albumen was substituted for the KNO_3 there was no visible variation in the fungous growth. When urea salts were substituted for the KNO_3 there was a more abundant growth of mycelium, the hyphae were peculiarly twisted, and many conidia were produced.

When either $CuSO_4$, $HgCl_2$, $ZnSO_4$, tartaric acid, or phenol were added to Richard's solution at the rate of 1 gr. to 1,000 cc. there was no growth of the fungus.

EFFECT OF VARIOUS VOLATILE SUBSTANCES ON THE VEGETATIVE GROWTH OF THE FUNGUS

Cultures of the peony *Phytophthora* were grown on lima bean agar in petri plates until a diameter of 25–30 cc. was reached. The petri dish cultures were then placed in culture-dish chambers and a small piece of cotton placed at the side of the petri dish. On the cotton were placed a few drops (not to exceed 3 cc.) of a volatile substance, the lid was removed from the petri plate, and the culture-dish chamber was then covered. After a period of 60 hours at room temperature, the increase in the diameter of the colony was noted and the colony was also examined under the low power of the microscope to determine the effect of the substance on the vegetative growth. The lids were then placed on the petri dishes and the cultures allowed to stand 48 hours longer to determine whether the fungus was still alive. These experiments were carried on in such a way as to guard against contamination as much as possible, and very little contamination occurred.

The hyphae which were exposed to the vapors of acetone and of 25 per cent alcohol were much curled (Fig. 3). When oil of bergamot and menthol were used, there were many short, club-shaped branches of the mycelium. Turpentine caused the aerial hyphae to curl, and the submerged hyphae were club-shaped, many bursting at the end. Oil of eucalyptus caused rounded, knob-like swellings on the hyphae. Thymol caused a profuse branching of the hyphae at their tips. Clove oil caused the hyphae to be sinuous. The fungus was found to be dead in all cases except when thymol and clove oil were used.

When the colonies were kept in a chamber containing illuminating gas, the mycelium developed many warty protrusions and was finally killed. In CO_2 the fungus was profusely branched, but after being removed from the gas continued to grow. However, during the 4 days the culture was kept it failed to produce oospores, but did produce conidia in abundance. This was of interest since the fungus usually produces oogonia and antheridia upon the new growth.

THE EFFECT OF CERTAIN COLORS OF LIGHT ON THE DEVELOPMENT OF THE FUNGUS

Cultures of the fungus two days old were placed in light of different wave lengths obtained by the use of ray filters and left there for four days. The ray filters used were as follows: red, transmitting red only; yellow orange, having a high transmission of red and green with a sharp cut off beyond blue green; amber, absorbing the blue and ultra-violet; blue, transmitting approximately primary blue; bluish tint, transmitting a full spec-

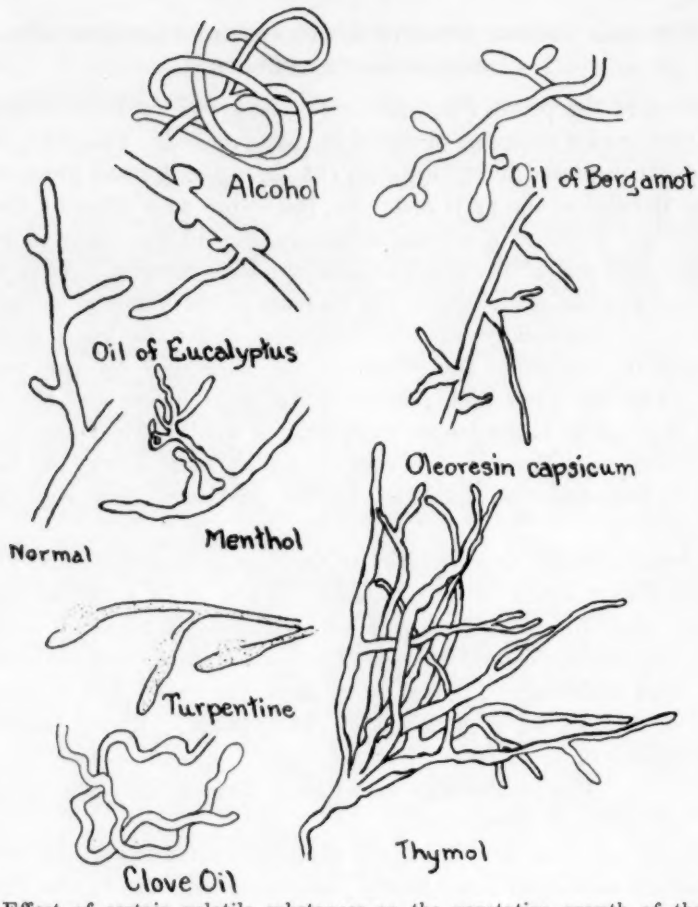

FIG. 3. Effect of certain volatile substances on the vegetative growth of the fungus.

trum but absorbing the infra red; smoky violet, completely removing the yellows; and canary, transmitting the full spectrum. The vegetative growth was about the same in all cases. When the red and yellow-orange filters were used, no conidia or oospores were produced; whereas they were produced in abundance under all the other filters tested.

THE PHENOMENON OF STALING AND INHIBITION

There seemed to be no definite staling effect of the colony of the peony *Phytophthora* upon the surrounding medium since the colony appeared to grow without any apparent diminishing rate. Leonian (3) found no staling phenomena in the *Phytophthora* species he studied. When two colonies of the peony *Phytophthora* were grown upon opposite sides of a petri plate,

the colonies grew together, the hyphal strands intermingled, and there was seemingly no inhibition. However, if *P. erythroseptica* was grown on one side of the plate and the peony *Phytophthora* on the other, there seemed to be a distinct antagonism. The hyphae did not intermingle and a strip of agar 1–2 mm. wide remained clear between the colonies. Along either side of this strip the fungi produced conidiophores instead of the usual oogonia and antheridia (Fig. 4). This agrees with the results obtained by Porter (7) when working with other fungi.

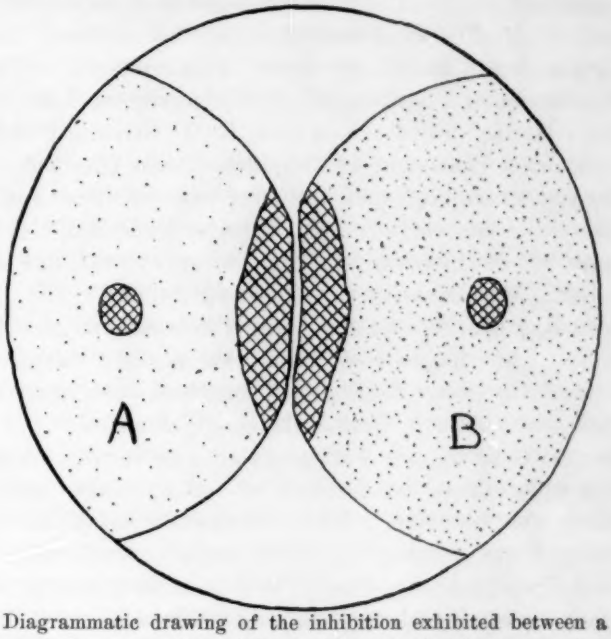

FIG. 4. Diagrammatic drawing of the inhibition exhibited between a colony of *P. erythroseptica* and the peony *Phytophthora*. The cross-hatched areas show the portions of the colonies in which conidia were formed. A. *Phytophthora* from peony. B. *Phytophthora erythroseptica*.

Since conidia are produced in abundance on the older portions of the colony, on the advancing mycelium when the surrounding agar is cut away, on the advancing mycelium when two colonies of different species approach each other, and when the fungus is grown on washed agar, it would seem that the production of conidia is stimulated by the absence of nutrient material.

COMPARISON OF THE PEONY PHYTOPHTHORA WITH OTHER PHYTOPHTHORA SPECIES

After the study of the peony fungus was begun, a number of the described species of *Phytophthora* were brought together for comparison.

Cultures of the following species were procured:

P. parasitica Dastur was obtained from J. B. Kendrick, Purdue University; *P. terrestris* Sherb., *P. colocasiae* Rac., *P. cryptogea* Pethyb. and Laf., *P. fagi* (Hartig) Hartig, *P. pipi* Leonian, *P. mexicana* Hotson and Hartge, and *P. palmivora* (Butl.) Butl. were obtained from Leon H. Leonian, Morgantown, W. Va.; *P. erythroseptica* Pethyb. was obtained from G. H. Pethybridge, England, and from the Centraalbureau, Baarn, Holland; *P. richardiae* Buisman was also obtained from Holland; a *Phytophthora* causing stump root rot of lily and another isolated from cotton boll were obtained from C. M. Tucker, Porto Rico; a *Phytophthora* from *Hevea*, one from abaca, and Reddick's tomato *Phytophthora* were obtained from the Department of Plant Pathology, University of Wisconsin; *P. infestans* (Mont.) DeBary was obtained from R. W. Samson, Purdue University, and *P. cactorum* (Lebert and Cohn) Schröt. was obtained from M. W. Gardner, Purdue University, and was also isolated from apples by the writers. Because of the very different type of their mycelial growth on lima bean agar, all of these except *P. cactorum*, *P. fagi*, and *P. erythroseptica* were distinctly different from the peony fungus.

On all the solid culture media, the peony *Phytophthora* grew better than did *P. cactorum*. As compared with the loose, cottony tufted appearance of the cultures of the peony *Phytophthora* on lima bean agar, the culture of *P. cactorum* had a smooth, thick, velvety appearance.

When the growth of the peony *Phytophthora* on various vegetable plugs was compared with that of *P. cactorum* on similar plugs, interesting cultural differences were observed. While the peony *Phytophthora* grown on sterilized peony stems produced a thick aerial growth with numerous oospores and a few chlamydospores, *P. cactorum* on a similar substratum produced a very scanty mycelium with only a few oospores. On the other hand, on apple tissue and on green bean pods, *P. cactorum* produced numerous hyphae and abundant oospores as compared with the slight aerial growth produced by the peony *Phytophthora* and its complete absence of spores. On the tomato plugs the mycelial growth of the two fungi was similar except that the peony *Phytophthora* produced oospores and conidia to a much greater extent than did *P. cactorum*. In order to check these results, the work was repeated, and a series of cultures of *P. erythroseptica* was used for comparison. It was found that *P. erythroseptica* was more like the peony *Phytophthora* in the amount of mycelial growth and spore production than was *P. cactorum*.

It is of interest to note that *P. cactorum* caused a complete rotting of both green and ripe tomatoes when the inoculum was placed over an injury. *Phytophthora parasitica* was able to penetrate the uninjured fruit

whether green or ripe, whereas the peony *Phytophthora* attacked only the ripe fruit and then only through an injury.

The peony fungus caused injured Grimes apples to decay but did not enter through the unbroken epidermis, whereas *P. cactorum* readily penetrated through the uninjured surface. The lesion on the apple caused by the peony *Phytophthora* did not have the zonate appearance characteristic of the lesions caused by *P. cactorum*. Upon examination of the apple tissue infected with *P. cactorum*, it was found that the fungus sends into the host cells long, finger-like haustoria similar to those described by Rosenbaum (11). In the apple tissue invaded by the peony *Phytophthora* the haustoria were similar to those which this fungus produced on the peony tissue (more or less spherical) but distinctly different from the haustoria of *P. cactorum*.

Inoculation of the peony was attempted by using *P. erythroseptica, P. parasitica, P. richardiae, P. fagi, P. cactorum, P. terrestris* and the *Phytophthora* causing stump root rot of lily. In no case did the fungus enter through the unwounded tissue. When inoculation was again attempted by injuring the surface on which the inoculum was placed, *P. cactorum* was the only one of these species that entered the plant and it caused merely a small lesion on the side of the stalk (Fig. 5, B).

All of the described species of the genus *Phytophthora* have many characters in common, so that it is necessary to consider each of them before attempting to determine the taxonomic position of the peony *Phytophthora*. At the start such species as *Phytophthora omnivora* DeBary, *P. erythroseptica* Pethyb., *P. infestans* (Mont.) DeBary, *P. phaseoli* Thaxt., *P. parasitica* Dastur, and others having a so-called amphigynous relationship of the sexual organs, can be set aside. *Phytophthora jatrophae* Jensen, *P. faberi* Mont., and *P. nicotianae* Breda de Haan are also eliminated because of their dense aerial growth and the absence of sexual organs on lima bean agar. This would leave among the described species *P. fagi* (Hartig) Hartig, *P. syringae* (Klebh.) Klebh., and *P. cactorum* (Lebert and Cohn) Schrot, as being more or less similar to the peony *Phytophthora*.

Phytophthora fagi differs from the peony fungus in that it produces on lima bean agar a uniformly dense white aerial mycelium. The conidia are more elongated and have a constant difference of 8–10 μ between the length and width and also have much more prominent apical papillae.

P. syringae differs distinctly from the peony *Phytophthora* in that the conidia are described as having various shapes, the length sometimes being twice the width. According to Rosenbaum (11) the submerged hyphae are much twisted and gnarled on potato agar and there is an absence of spore forms on lima bean agar. The peony *Phytophthora* on potato agar produced spores and normal mycelium.

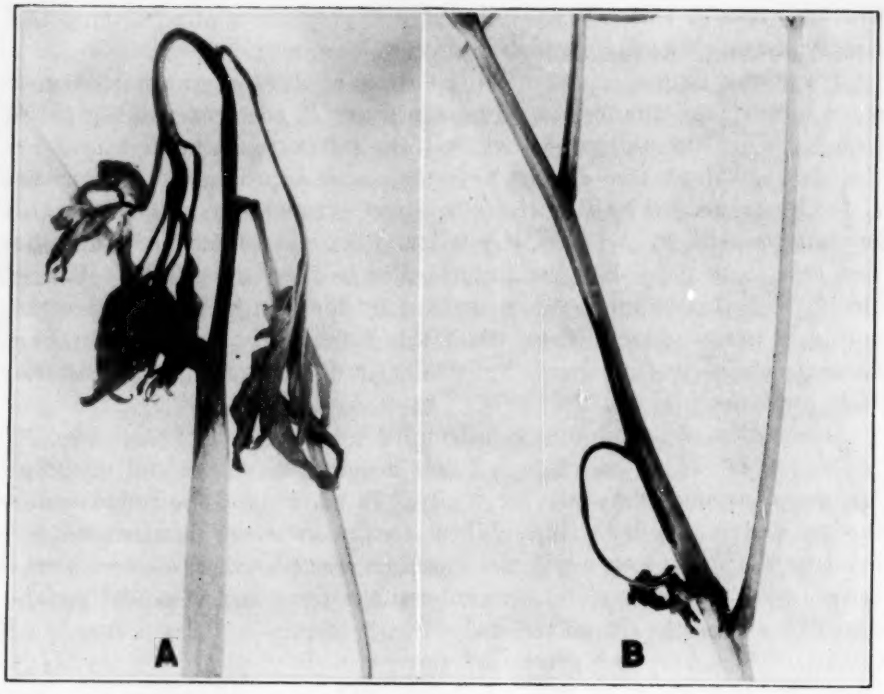

FIG. 5. A. Tip blight of peony caused by the peony *Phytophthora* one week after inoculation. B. Lesion on a peony stem produced by *P. cactorum* after three weeks.

The morphological differences are less marked between the peony fungus and *P. cactorum*. The long twining stalks of the oogonia and antheridia that are found in the peony *Phytophthora* are not found in *P. cactorum*. The apical papillae of the conidia of *P. cactorum* are prominent, whereas those of the peony fungus are broad and more or less flattened. The difference between the average lengths and widths of the conidia of *P. cactorum* is 9–11 μ, whereas the average difference for the peony *Phytophthora* is 4–6 μ. In culture the aerial mycelium of *P. cactorum* is uniformly dense while that of the peony fungus is in scattered, loose, cottony tufts. In its host tissue the peony *Phytophthora* produces small, more or less spherical haustoria instead of the long finger-like projections of *P. cactorum*. All attempts to inoculate peonies with *P. cactorum* have failed to cause the typical *Phytophthora* blight.

These facts indicate that the peony *Phytophthora* apparently is an undescribed species, and the name *Phytophthora paeoniae* n. sp. is proposed.

Phytophthora paeoniae n. sp.

Mycelium at first continuous and granular, somewhat geniculate, later becoming vacuolate and finally sparingly septate, 7–10 μ in diameter, intercellular, producing small sub-spherical haustoria; conidiophores not distinguishable from the mycelium; produced on mycelium of colony from 4 to 5 days old; bear from 1 to 15 conidia; conidia terminal on short unbranched lateral hyphae, normally ovate and papillate, the papillae being usually broad and flat; conidia measure 22.5–35.6 μ by 26.2–41.2 μ, mostly 26–28 μ by 31–33 μ, germinating by zoospores, sometimes by germ tube; zoospores biciliate, 8–10 μ by 10–12 μ, becoming globose, and 8–10 μ in diameter; chlamydospores few, 30–40 μ in diameter; oogonia in abundance on mycelium from 24 to 48 hours old and older, 26–34 μ in diameter, pale to brownish in color; antheridium oval to globose, closely appressed to base of oogonium at side of oogonial stalk; oospores globose, thick walled, 24–30 μ in diameter; formed profusely on most culture media.

Parasitic on the tips, stalks, and leaves of peony in Indiana, Pennsylvania, Connecticut, and Kansas, United States of America.

SUMMARY

1. The Phytophthora blight of peony is primarily a tip and stem blight, but often appears as a leaf spot. The infected portions turn black and wither.

2. Inoculation experiments with pure cultures show that the peony stalks are readily blighted by this fungus. Reisolations were made. Inoculations were attempted with eight other species of *Phytophthora* but without success.

3. The mycelium is intercellular with more or less spherical haustoria extending into the cells of the host tissue. It was found in the cortex, pericycle, medullary rays, and pith. No spores were found in the host tissue.

4. The oogonia and antheridia are produced on long hyphae, the antheridial stalk often twisting about the oogonial stalk.

5. The conidia are much shorter and broader than those usually found in the genus *Phytophthora*.

6. Chlamydospores have been found only in cultures on sterilized peony stems.

7. The optimum temperature for the growth of the fungus is between 20° and 26° C. The minimum is about 14° C. and the maximum about 34° C.

8. Peculiar morphological reactions to the vapors of various volatile substances tested were observed in cultures of this fungus.

9. Spores were produced in all lights tested except under red and yellow-orange filters.

10. The fungus grew best on a culture medium having a pH of about 6.6. It grew better on a medium having a pH of 8.4 than on a medium with a pH of 5.2.

11. Growth of the fungus was not inhibited by its staling products.

12. Although a colony of the peony *Phytophthora* was not inhibited by another of the same species, yet there was marked inhibition between this fungus and *P. erythroseptica*.

13. The peony *Phytophthora* differs from previously described species, and particularly from *P. cactorum* which it most nearly resembles, in cultural characters, shape of conidia and haustoria, length and twining character of the antheridial stalk, and pathogenicity. It has been described herein as *Phytophthora paeoniae* n. sp.

The writers feel deeply indebted to Dr. M. W. Gardner, of the Agricultural Experiment Station, for his generous assistance during the course of this investigation and to Professor E. J. Kohl, who made the photographs and photomicrographs.

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EXPLANATION OF PLATE XXIX

Camera lucida drawings of *Phytophthora paeoniae* n. sp. (×400)

A. Intercellular mycelium in peony stem with small, more or less spherical haustoria extending into the cells of the host tissue.

B. Typical conidiophore bearing conidia. 1. Conidia germinating by production of swarm spores. 2. Conidium germinating by production of germ tubes. 3 and 4. Germinating zoospores.

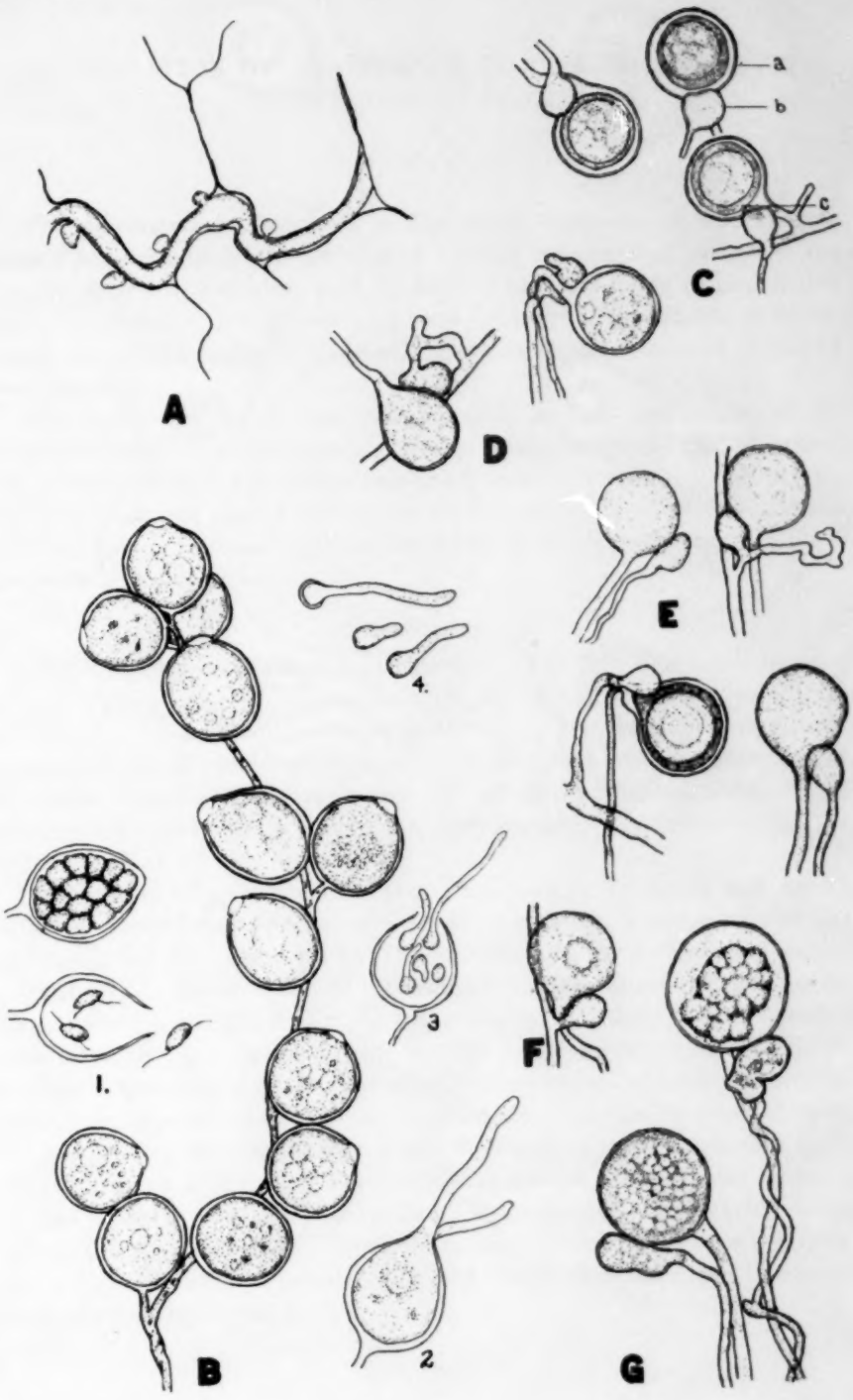
C. Three mature oospores showing relation of oogonium (a) to antheridium (b). Note fertilization tube (c).

D. Two oogonia and antheridia before fertilization. The figure at the right shows how the antheridium sometimes has a tendency to surround the oogonial stalk.

E. Four oogonia and antheridia in early stages of development.

F. Early stage showing antheridium closely appressed to base of oogonium.

G. Oogonia and antheridia showing their relatively long supporting hyphae. The right-hand group shows how the antheridial stalk oftentimes entwines around the oogonial stalk.



UNIV. OF MICH.

LOCALIZATION OF RESISTANCE TO POWDERY MILDEW IN THE BARLEY PLANT

J. R. MACKIE

The investigations described in this paper represent an endeavor to delimit more definitely the features of varietal resistance of barleys to the attacks of powdery mildew, and an inquiry into the likely causes of this resistance with the view of obtaining some light on the probability of breeding or selecting a barley permanently immune to the attacks of *Erysiphe graminis* D.C.

The author undertook some morphological and cultural studies of the fungus collected in several localities in California, but could find no noticeable differences from the mildew previously described on barley. However, the severe damage caused by mildew during the last few years could not be overlooked. The loss sometimes included the whole grain crop, the fields having to be cut for feed.

THE DISEASE

The symptoms of the attack are marked. The first sign is the appearance of white flecks of mycelium upon the leaves, which increase rapidly in size and are covered with powdery conidia in a few days. There follows a gradual but complete yellowing of the whole plant with the exception of the areas immediately surrounding the infected epidermal cells. These spots remain green for a considerable period after the yellowing of the rest of the leaf.

A cessation of growth results at a time (usually in March and April) which is normally the period of greatest vegetative development for the plant, *i.e.*, the tillering period. The yellowing of the entire plant points suggestively to the secretion of a substance of toxic nature by the fungus. Soon, however, the plant sends out new leaves, and these, appearing at a time when the drier and brighter weather tends to suppress the mildew, manage to retain their vitality, although scattered spots of infection develop. Notwithstanding this new growth, the plant never regains its original vigor, but is smaller than normal, with fewer tillers, since many die before heading. Maturity is delayed. The heads that mature contain many abortive kernels at both basal and apical ends, those kernels that develop being shriveled and poorly filled. Toward the end of ripening there is a darkening in the region of the germ. Since the fungus does not attack the head, these effects are indirect.

The injuries mentioned above destroy the export value of the crop and put it in the feed, or low price, class.

THE MILDEW FUNGUS AS OBSERVED ON BARLEY

In the Erysiphaceae the germinating conidium forms a flattened appressorium against the wall of a cell of the appropriate host (usually an epidermal cell), and from this a slender infection thread grows down into the cell. A reaction, supposedly enzymatic, takes place around the point of this infection thread making a circular depressed area which in many cases remains colorless in stained preparations (Figs. 1 and 3). An outgrowth

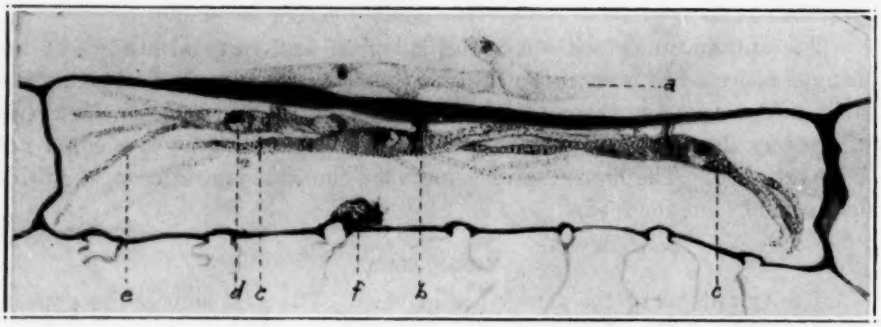


FIG. 1. Eight-day culture of mildew on Atlas barley. Showing superficial mycelium (a), and infection thread (b), several large haustoria (c) with prominent nuclei (d) and lobed ends (e). The host nucleus (f) is not involved. (×400)

or swelling of the inner layer of the host cell wall accompanies the penetration of the thread, and after penetration surrounds it like a collar, frequently becoming a granular or disintegrated layer about the haustorium. In *Erysiphe graminis* the haustorium is branched at one end, or more characteristically at both ends, into several finger-like lobes. There is a single central nucleus in the main body of the haustorium. (Fig. 3.) Mycelium development in this species is external and chains of conidia are produced on erect branches from the mycelium, while the perithecia are formed partially imbedded in it. Some genera and species of the Erysiphaceae, however, are endophytic.

The assumption of early mycologists that morphological identity delineates a species has been modified by the discovery that within a single morphological species of a parasitic fungus there may be several physiological strains, or forms, which usually can be distinguished only by their cultural behavior.

In the Erysiphaceae the specialization is found both in ascopores and conidia (8).

SUSCEPTIBILITY AND RESISTANCE TO MILDEW

Resistance and susceptibility are variable qualities. The susceptibility of a host to a given physiological form of infecting organism has been noted to vary with change of locality, and to be different in different years and seasons. Since this is the case, physiological specialization can be said to hold strictly only under a definite set of conditions with a sufficiently large number of trials to assure uniformity.

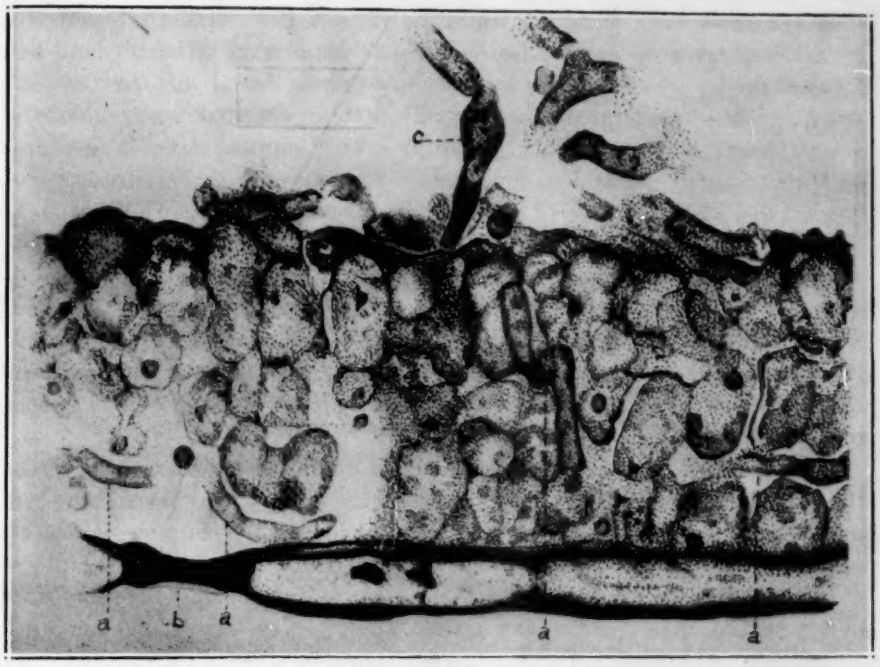


FIG. 2. Eight-day culture of mildew on exposed mesophyll of ventral surface on Hero barley. Showing intercellular hyphae (a), stoma of the host (b), and conidiophore (c) growing from scraped surface. (×400)

All gradations seem to exist between true susceptibility and absolute resistance, and at least three types of reaction between mildew and its hosts have been observed. Neger (5) showed that on resistant hosts the conidia of the mildew germinate, but soon die without having caused penetration of the host tissue. On other host plants haustoria are produced, but the host cells become filled with gum, stopping the growth of the parasite. If occasionally a haustorium escapes and continues to grow, this constitutes what Salmon, according to Neger (5), terms "subinfection." Salmon (12) found that the disintegration of the haustoria of *Erysiphe graminis* occurred in from one to six days when they developed in unsuitable hosts.

The third type of infection is that in which the mycelium and haustoria develop normally, producing abundant conidia in from five to eight days.

Morphological and anatomical features of the host plants which might be thought to encourage or ward off fungus penetration at once suggest themselves as possible factors in the problem of immunity. There are certain evidences both for and against the assumption.

In working with *Erysiphe cichoracearum*, Reed (7) noted that two of three cucurbits resistant to the fungus had considerably rougher surfaces than the susceptible varieties, while the third had an epidermis protected by a heavy layer of wax. Also, Neger (4) found that although both surfaces of the leaves of *Quercus pedunculata* (whose dorsal surfaces possess a much thicker cuticle than the ventral) were inoculated with conidia of *Microsphaera alni* under equal conditions, only stunted mycelia appeared on the dorsal surface and no appressoria were formed, while normal development of haustoria occurred on the ventral surface. Salmon (13) was not able to get mildew on untreated mature leaves of *Euonymus japonicus*, whereas the young leaves became infected. A similar condition was observed in the case of apple and oak mildews by other workers. On the other hand, Neger (3), working with *Erysiphe cichoracearum*, observed only subinfection on the host (*Sonchus oleraceus*) during the summer, while toward the end of the host's growth period the development of the fungus was complete and normal.

It is interesting to note that Gassner (1) found that the season governed the susceptibility of barley plants of the same variety at different stages of growth, the more mature plants as well as the young being susceptible to mildew in the summer, while in spring and winter only the young plants were affected. This agrees with observations in California where late-sown barley suffers most from mildew attack.

Salmon (11) performed many experiments in which host plants known to be immune under the conditions of growth became infected with mildew after the host tissue had been disturbed by removal of a portion of the epidermis with a knife or a hot iron point. This type of infection is termed by him "xenoparasitism." This might indicate that resistance is a mechanical matter, were it not for other experiments of Salmon's in which the epidermis was left intact, but the general vigor of the host reduced by treatment with ether, chloroform, or alcohol.

There is some disagreement among authors as to the relation between turgor of host cells and infection. Rivera (9, 10) is quoted as getting no attack by *Erysiphe graminis* on plant organs which are turgid, owing, perhaps, to the mechanical resistance of the turgid cell walls. This does not seem clear, since the experience of the author and of many other workers

with damp chambers is that turgidity is favorable and that infection does not occur readily in limp or yellowed leaves.

Vavilov (14, 15) found no correlation between susceptibility and osmotic pressure or acidity of sap. Others found the average concentration slightly greater in resistant plants. Owing to the difficulties involved in making these tests the results must remain somewhat in doubt. Vavilov found that plants immune to the parasite stayed so in spite of various treatments with nitrogenous fertilizers. Many other experiments were performed, and the general opinion seems to favor the view that the higher the rate of assimilation in the host the greater the success of the fungus.

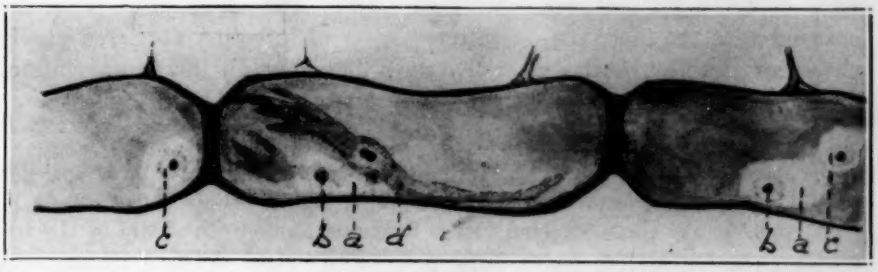


FIG. 3. Epidermal cells of Atlas barley showing spots (a) decolorized by the infection threads (b). The depressed areas (c) surround the threads. The haustoria (d) are seen through the wall of the host cell. (×400)

EXPERIMENTS WITH SUSCEPTIBLE AND RESISTANT BARLEYS

Eight varieties of *Hordeum vulgare* were chosen for these tests after preliminary field inspection. These were Atlas, C. I. 2118; Sacramento, C. I. 4108; Hero, C. I. 1286; Chile, C. I. 3482; Goldfoil, C. I. 928; Hanna, C. I. 1122; Oderbrucker, C. I. 957, and Common Chile, C. I. 663. The first four varieties listed were obtained from the California Agricultural Experiment Station and the last four from Dr. E. B. Mains, of Purdue University Agricultural Experiment Station, LaFayette, Indiana.

Cultural experiments were undertaken by the author to determine the relative susceptibility and resistance of the chosen varieties of barley under the conditions of culture. The rating for degree of infection with a scale from 0° for highly resistant to 4° for very susceptible was followed as used by Mains (2).

The stock cultures of the physiological form of mildew specialized for barley species obtained from an infected field were kept in the greenhouse under a glass and cheesecloth cage. Transfers were made about every three to four weeks. The varieties used as hosts were Hero and Hanna. The inoculations were made with single conidia by means of a fine glass rod.

The inoculated plants were kept for four days under a bell jar, then placed under the cage. Abundant conidia formed on these plants and were used as the stock source for the following experiments.

FIELD TESTS

1. Early in February, 1927, Atlas, Sacramento, Hero, and Chile were sown in double rows in the field, and after the appearance of the fourth leaf sprayed with sterile distilled water and then with a fresh suspension of ripe conidia. These plants were not covered. Both Atlas and Hero developed mildew patches by the fourth day, conidia appearing in six days. No mildew developed on Sacramento, but after a week several small patches were observed on scattered plants of Chile. The check rows were not sprayed with the inoculum. Scattered spots of infection appeared on the checks of Hero and Atlas after five days, but none on the Sacramento or Chile. Sacramento being slow growing, another inoculation was made on it two weeks later, but no infection occurred.

2. Later, in March, all eight varieties were planted in 8-inch pots, three plants to the pot. They were germinated in the greenhouse until the hypocotyl was visible, then removed to the field and sunk to the level of the soil under close cheesecloth cages, four pots to the cage. These plants were watered carefully so that no water (containing substances possibly toxic to mildew) splashed upon the leaves. After the appearance of the fourth leaf on all varieties, the leaves were sprayed with sterile distilled water, and three leaves on each plant were inoculated by means of a scalpel sterilized in alcohol with conidia from the stock plants. (A pot of each variety was kept as check under a separate cage, but no mildew developed on any of these checks until after the removal of the cage.) The conidia were applied $1\frac{1}{2}$ inches above the top of the leaf sheath in each case and the leaf marked by a bit of thread tied loosely about the base. The plants were allowed to grow for ten days under the cages. The cheesecloth was then removed on three sides to reduce the temperature. Examinations of the inoculated leaves were made frequently, and the following tentative rating given the varieties: Atlas 4°, Sacramento 0°, Hero 3°–4°, Chile 1°–2°, Goldfoil 0°, Hanna 4°, Oderbrucker 2°–4°, and Common Chile 0°.

3. Damp chamber experiments were carried out with all eight varieties under the same conditions of germination, age, washing, etc., as above. Single conidium inoculations were made on the leaves of plants grown in 6-inch pots, two plants to a pot. Three leaves were inoculated on one plant in each pot, the other plant being kept as a check. In no case did the latter become infected until after the removal of the jars. The inoculations were made with a fine glass rod which was touched to the spores of the stock culture, examined under a low power objective to be sure of one conidium, and

the conidium placed 1½ inches above the top of the sheath on moist, marked leaves. The plants were kept under a bell jar for five days, ventilation being secured through cotton placed between the slightly raised edge of the bell jar and the soil of the pot. At the end of this period the bell jars were removed and a careful examination was made of the inoculated leaves. What seemed to be subinfection occurred on both Chile and Common Chile. No infection appeared on Sacramento or Goldfoil. All the others developed a moderately heavy growth of conidia which increased rapidly after the removal of the bell jars. True infection appeared on the Chile plants in three cases after the removal of the jars.

4. Petri dish tests were made with all eight varieties. Two series were run for each variety, one containing two leaves placed ventral surface down, and the other containing two leaves ventral surface uppermost. The petri dishes had been sterilized in a steam autoclave for one-half hour at 15 lbs. after four layers of paper toweling and considerable water had been placed in them. The leaves, while on the plants, were washed in sterile distilled water and portions of two from each plant were cut off near the center of the blade and placed with sterile forceps in a petri dish with the corresponding surface uppermost in both. In each case one of the pieces of leaf was inoculated by a sterile scalpel with several conidia, the other piece being left as a check. In no case did the check show mildew infection, although in two cases saprophytic fungi started to grow at the cut edges after about five days. The dishes were placed where they got direct morning sunlight and indirect afternoon light. After four days brownish spots had appeared on the dorsal surface series of Common Chile beneath the spot of inoculation, but no other indications of growth were apparent. Mycelia appeared on the dorsal surface series of Chile, but not on the ventral. No infection was obtained on Sacramento or Goldfoil. True infection with conidial formation appeared on the inoculated spots on both surfaces of Atlas, Hero, Hanna, and Oderbrucker. As a result of these studies the rating of the varieties was considered to be: Atlas 4°, Sacramento 0°, Hero 3°–4°, Chile 1°–3°, Goldfoil 0°, Hanna 4°, Oderbrucker 2°–4°, and Common Chile 0°–1°.

MICROSCOPICAL STUDIES

1. A series of tests (series 1–7) was run to determine the best conditions for fixing, sectioning, staining, etc. Atlas and Hero were used for these preliminary tests and the leaves were taken from three to nine days after inoculation. Two formulae for fixing solutions were tried, the material being kept in these for three days at room temperature, 33° and 32° F. Uninoculated leaves were also run under the same conditions. One fixative

used was the Wisconsin[1] (one-half strength). This seemed to hold the shape of the host cells better than the second fixative, but the sections were more difficult to stain. The second fixative[2] seemed the more satisfactory. Sections 12 μ in thickness were found to be best, although they were good down to 10 μ. Various stains were tried, but the triple stain (saffranin, gentian violet, and orange G) gave the best results. On the whole, leaves fixed in the second solution at 32° F., cut 12 μ thick, and stained in triple stain gave the most satisfactory results.

2. Sections were made of all eight varieties (series 8 and 9), 12 μ thick, a series being fixed in each fixing solution at 32° F., and triple stained. These were used for study of the method of infection.

3. Sections of the petri-dish cultures were made (series 10) for the dorsal and ventral surfaces with checks as above, the difference here being that before inoculation a small portion of the epidermis on the opposite side of the leaf had been scraped away with a sharp sterile scalpel and the cut surface immediately placed in contact with the wet toweling in the dishes. The inoculations were made on the uninjured epidermis over the cut. (The check leaves were cut but not inoculated, and none were infected.) After four days infection had occurred on all except Goldfoil. Haustoria appeared to be formed in all cases. The tests were not continued longer.

4. Another series of inoculations was made on the cut surfaces (series 11). Cuts similar to the above were made on all of the eight varieties on leaves attached to the plants, marked as before, and the plants kept under bell jars. Uninoculated check cuts were made on the same plant. In no cases were these infected.

Mycelia were formed within the mesophyll in all except Hanna, Chile, and Common Chile (Fig. 2). No reason suggests itself for the failure in these cases, since this series was repeated with similar results. These cultures were fixed, after 8 days, in the second fixative at 32° F. Sections were cut 12 μ thick and triple stained.

5. A similar series with cuts on both dorsal and ventral surfaces was made (series 13). No apparent distinction was observed. These were fixed after eight days and were treated in all respects as above.

6. No morphological peculiarities in the resistant varieties were noted from examination of slides. The size of spines and of cells varied more within the variety, depending on the age of the leaf, than between varieties. The same circumstances were found in the matter of cuticle thickness in sections stained with Sudan III, according to Priestley's (6) formula.

[1] 100 cc. 50 per cent alcohol
6.5 cc. formalin
2.5 cc. glacial acetic acid.

[2] 1 gm. chromic acid
1 cc. glacial acetic acid
5 gm. salts of urea
In 200 cc. distilled water.

SUMMARY

1. Susceptibility of a variety of barley to a given suitable physiological form of mildew seemed fairly constant under the conditions of culture.

2. No mean morphological differences in leaves were observed between varieties which differed in susceptibility to the given strain of mildew.

3. Injury to the host tissue by removal of epidermal cells seemed in all cases but one to reduce the resistance of the host and to allow at least sub-infection.

4. When the inoculations were made on the exposed mesophyll resistance was destroyed in all cases, and in the majority endophytic mycelium was developed.

5. The penetration of the infection threads seemed normal, a decolorized area appearing around the point of penetration in the stained sections. The collar, or membrane, formed by the host cell about the penetrating hypha, appeared clearly, while no sac or disorganized membrane was observed about the haustorium. The haustoria were typically lobed at both ends, uninuclear, and frequently many occurred in a single epidermal cell.

6. In no case was the mildew observed to enter at a stoma, whereas the mycelia commonly passed across the opening and haustoria occurred in the epidermal cells on either side.

7. The varieties of barley studied were found to have the following degrees of infection under the conditions of culture rated according to Mains' scale: Sacramento 0°, Goldfoil 0°, Common Chile 0°–1°, Chile 1°–3°, Oderbrucker 2°–4°, Hero 3°–4°, Hanna 4°, and Atlas 4°.

It seems, therefore, that the resistance of a variety of barley may be quite stable toward the given physiological form of mildew under these conditions. It seems possible then, for any given locality, to obtain a variety of barley which under ordinary conditions will be immune to *Erysiphe graminis* D.C. This resistance may be altered by circumstances affecting the vitality of the host tissue, such as a mechanical injury. It appears unlikely that this "xenoparasitism" would be able to cause the general infection of an immune variety.

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THE EFFECT OF SEED DISINFECTANTS ON SMUT AND ON YIELD OF MILLET

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Millet, *Setaria italica* (L.) Beauv., is a crop of major importance in the northern and northeastern provinces of China as well as in Manchuria. Crop disease surveys in the summer of 1925, and again in 1926, indicated that kernel smut caused by *Ustilago crameri* (Korn.) is one of the important limiting factors in the production of millet. It was not uncommon to find fields where the smut ranged from 10 to 25 per cent, and in a few cases over 50 per cent of the heads were infected. Seed from such badly infected fields together with clean seed offered excellent material for measuring the relative efficiency of different fungicides in controlling smut, the effect of these fungicides on the yield, and the losses resulting from heavy smut infection. When this study was initiated it was not known whether the remedies which had been used in the United States would be effective in China, where environmental conditions, varieties, and cultural practices are markedly different.

Experimental data concerning millet smut control and yield increases are very limited. Vasey[2] tried different concentrations of formaldehyde and found that a solution as dilute as 1 part of formaldehyde to 640 parts of water would kill the smut spores. He recommended either sprinkling or soaking of millet seed, using one part of 40 per cent formaldehyde in 320 parts of water. Melchers,[3] in a recent paper, reported that copper carbonate at the rate of four ounces a bushel was more economical than any other dust and gave almost perfect control of millet smut. Stone[4] states that both the formalin sprinkle and soak prevented germination of millet seed, and the dry formaldehyde also checked germination. He found that

[1] At the time these investigations were carried on the senior author was plant pathologist at the University of Nanking, Nanking, China.

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[2] Vasey, H. E. Millet smuts and their control. Colo. Agr. Exp. Sta. Bul. 242. 1918.

[3] Melchers, L. E. Studies on the control of millet smut. Phytopath. 17: 739. 1927.

[4] Stone, R. E. Treatment of millet seed to prevent smut (Abst.). Phytopath. 18: 479. 1928.

Semesan and Uspulun solutions, each 0.25 per cent strength, Dupont No. 12 dust, and copper carbonate reduced smut to 1.9, 0.9, 0.6 and 1 per cent respectively. Untreated seed had 56 per cent smut.

There seems to be no published information dealing with the effect of different fungicides on the yield of smutted seed. This fact led to an investigation of this aspect of the seed treatment problem. In making a comparative study of the yield of treated and untreated seed it seemed desirable to use a number of disinfectants available and to determine their relative merits in controlling smut.

EFFECT OF SEED DISINFECTANTS ON MILLET SMUT

In the spring of 1926 a series of seed treatment experiments were begun to determine the relative efficiency of dry and liquid disinfectants. The seed used in the test was naturally smutted seed secured from a farmer whose field had 50 per cent of the heads infected with *Ustilago crameri* in 1925. The seed was collected after the threshing operation. The chemical disinfectants used in the experiments in 1926 were cold formaldehyde (1–320), Uspulun solution (3 grams a liter of water), dry Uspulun, dry Tillantin "B," and copper carbonate containing 54 per cent metallic copper. The seed was soaked two hours in the formaldehyde solution and one hour in the Uspulun solution. After soaking, the seed was spread out to dry. The rate of application for all of the dust treatments was one gram to a pound of seed, which is approximately 2 ounces a bushel. For each treatment an equal quantity of seed (60 grams) was placed in a flask, the required amount of dust added, and the flask shaken for at least five minutes or until the dust was evenly distributed on the seed. Later, 10 envelopes each containing a carefully measured quantity of seed equal to about 6 grams were prepared from each treated lot of seed. In the case of the liquid treatments the seed was weighed before treatment and after it was dried, due allowance being made for increase in size and weight.

The experiment consisted of 91 rows, each 16 feet long, arranged in such a way that every third row was a check. In this way each pair of rows from treated seed was between two rows from untreated seed. Seed from smut-free heads was included as one of the treatments. This plan made it possible to compare each treated row either with a check next to it or with an average of two checks. Each treatment was replicated 9 times, making ten in all, occurring in regular order in the plot. Border rows of common millet were planted on each end of the plot. The rows were one foot apart so that no cultivation was necessary.

The plot was planted on June 23 and harvested September 7, 1926. At harvest time each row was cut separately, the grain tied, and a label with the row number attached to it. Before threshing the total number of heads

per row was determined, together with the number of heads either partially or entirely destroyed by smut. Care was taken to prevent shattering. Each row was threshed separately and the weight of the seed determined in grams.

RESULTS IN 1926

A study of the records from this plot showed that the checks were quite uniform in yield, which indicated that the soil did not vary greatly in fertility. In computing the results, each treated row was paired with its adjacent check and a comparison made between each 10 pairs for a particular treatment. By the use of "Student's" method the reliability of the results was measured in terms of odds. A summary of the results for 1926 are presented in table 1.

The acre yield data shown in table 1 as well as the data on percentages of smut are averages of 10 in every case.

None of the treatments completely eliminated smut. This may be due to insufficient quantity of dust in the case of the dry treatments, although the formaldehyde soak treatment was even less effective than dry Uspulun and Tillantin "B." Furthermore, the soil on which the plot was planted had never grown millet before. In only three cases, however, was the percentage of smut in a treated row greater than its nearest check and all of these three were in the rows treated with Uspulun solution. Tillantin "B" and dry Uspulun each proved slightly more effective in controlling smut than any of the other disinfectants.

RESULTS IN 1927 WITH NATURALLY SMUTTED SEED

In the spring of 1927 the test was continued in much the same way as in the previous year except that the rows were 10 feet long and there were two separate plots planted, one with naturally smutted seed of the same source as in 1926, the other with seed artificially smutted. In the first plot, only three dusts were used, namely Uspulun, Tillantin "B," and copper carbonate, each at the rate of 2 ounces a bushel. A check was planted every fourth row so that the three treatments were between two checks. Nineteen replications were made, giving 20 rows of each treatment. The arrangement in planting was such that the row treated with Uspulun always occurred between the rows treated with copper carbonate and Tillantin. In computing the results each treated row was paired with an average of the two nearest checks and odds determined by "Student's" method. The results of this plot are shown in table 2.

The soil used in 1927 for the first plot was much more fertile than that used in the previous year. Differences were more uniform and with a

TABLE 1.—*Effect of certain treatments of naturally smutted millet seed on smut and on the yield. Nanking, China, 1926*

Treatment	Smutted heads (per cent.)		Difference in per cent	Acre yields (bu.)		Increase due to treament (bu.)	Gain in per cent	Odds
	Treated	Not treated		Treated	Not treated			
Selected heads	4.7	30.9	26.2	24.0	17.6	6.4	36.3	344–1
Formaldehyde	3.3	21.4	18.1	22.9	18.0	4.9	27.2	163–1
Uspulun solution	15.2	21.4	6.2	19.7	18.0	1.7	9.4	10.9–1
Uspulun (dry)	3.0	30.1	27.1	22.3	18.0	4.3	23.9	81–1
Copper carbonate	4.1	30.1	26.1	21.8	18.0	3.8	21.1	22.5–1
Tillantin "B"	2.7	27.1	24.4	23.9	18.4	5.5	29.9	908–1
Average of all checks		26.8			18.0			

TABLE 2.—*Effect of certain dust treatments of naturally smutted millet seed on smut and on yield. Nanking, China, 1927*

Treatment	No. heads per row	Smutted heads (per cent)		Difference in per cent	Acre yield (bu.)		Increase due to treatment (bu.)	Gain in per cent	Odds
		Treated	Not treated		Treated	Not treated			
Uspulun (dry)	228	3.4	20.6	17.2	48.6	36.9	11.7	31.7	4999 to 1
Copper carbonate	213	2.6	20.6	18.0	44.7	36.9	7.8	21.1	2499 to 1
Tillantin "B"	274	4.7	20.6	15.9	46.0	36.9	9.1	24.7	2499 to 1
Average of all checks	197		20.6			36.9			

larger number of replications the odds are greater. The yield of the checks was twice as great as in 1926, even though the percentage of smut is not much less. None of the treatments completely eliminated smut but all reduced it to less than 5 per cent as compared with 20.6 per cent in the checks. Dry Uspulun allowed 0.8 per cent more smut than copper carbonate but gave the largest increase in acre yield, namely 11.7 bushels. Dry Tillantin gave the poorest control of any of the treatments but produced an increase of 9.1 bushels an acre, which is 2.6 less than for Uspulun and 1.3 more than for copper carbonate.

RESULTS IN 1927 WITH ARTIFICIALLY SMUTTED SEED

In the spring of 1927 the quantity of naturally smutted seed available was insufficient for all of the tests. Accordingly a sample of nearly smut-free seed was inoculated with smut spores two years old. The smutted heads were rubbed between the hands, put through a fine screen and the resultant smut powder was scattered evenly over the seed by first placing a quantity of seed in a tea strainer and then adding the smut powder. A thorough shaking of the mixture distributed the smut spores over the seed. This seed was then divided into several lots and treated with four different dusts and two liquids. The dusts were used at the rate of 2 ounces a bushel. The two liquid treatments, Uspulun and Tillantin-Hoesht, were made up by dissolving 3 grams of the dust in a liter of water. The seed was soaked one hour and then thoroughly dried.

The planting plan was the same as for 1926, that is, two treated rows were placed between a pair of checks. The rows, however, were only 10 feet long, with nine replications of each treatment. This plot was planted at the same time as the one with naturally smutted seed. The results of this test are given in table 3.

The results presented in table 3 show that all of the treatments reduced the smut to less than 1 per cent, but the amount of smut in the checks was low as compared with that in plants grown from naturally smutted seed.

In the two years' work with millet smut eight different seed disinfectants were used on both naturally and artificially smutted seed. The old standard formaldehyde treatment proved just about as effective in controlling smut as any other treatment used, but it did not completely eliminate the disease on badly smutted seed. Uspulun and Tillantin "B" each used as dusts gave approximately the same control on the two kinds of seed. The average percentage of smut for the two years on naturally smutted seed was 3.2 when treated with Uspulun, 3.7 for Tillantin "B," and 3.4 for copper carbonate. If we make the comparison between the three dusts on the basis of the smut reduction due to treatment we find that there is little difference. The average percentage of smut reduction for the two

TABLE 3.—*Effect of certain dry and liquid treatments of artificially smutted millet seed on smut and on yield.*[a] *Nanking, China, 1927*

Treatment	No. heads per row		Difference	Smutted heads (per cent)		Difference in per cent	Acre yields bu.		Gain in bu.	Gain in per cent
	Treated	Not treated		Treated	Not treated		Treated	Not treated		
Uspulun (dry)	219	187	32	0.9	6.8	5.9	41.6	40.4	1.2	3.0
Uspulun solution	214	187	27	0.6	6.8	6.2	41.1	40.5	0.6	1.5
Tillantin-Trochenbeize	208	212	–4	0.3	6.8	6.5	44.2	41.3	2.9	7.0
Tillantin-Nazzbeize	208	212	–4	0.2	6.8	6.6	40.1	41.3	–1.2	–2.9
Tillantin-"B"	219	200	19	0.9	6.8	5.9	43.2	42.1	1.1	2.6
Tillantin-Hoesht solution	196	200	–4	0.4	6.8	6.4	41.9	41.7	0.2	0.5
Average of all checks		198			6.8			41.2		

[a] All of the materials used in this test were furnished by the China-Export-Import-Bank Co. Ltd., of Shanghai, China.

years on naturally smutted seed is 22.2 for Uspulun, 22.1 for copper carbonate, and 21.2 for Tillantin "B." Apparently the three dusts are about equally effective in controlling smut. It is possible that a heavier application in each case would come nearer to a complete control.

Uspulun solution gave practically as good control as any other material on artificially smutted seed, but in one year's test it was very ineffective when used on naturally smutted seed. Three other disinfectants were used only on artificially smutted seed, namely Trochenbeize and Nazzbeize as dusts and Tillantin-Hoesht as a solution. Each of these three gave slightly better control than any of the other treatments in one year's trial.

EFFECT OF FUNGICIDES ON THE YIELD OF SMUTTED MILLET SEED

The results presented in tables 1, 2, and 3 show some variation in the yields from smutted seed treated with different fungicides. In two instances the difference in the yield due to liquid and dry Uspulun could be attributed to more effective control of smut. For example, in 1926 the increase in yield following treatment with Uspulun solution was 1.7 bushels an acre with 15.2 per cent smut on the treated rows. Dry Uspulun reduced the smut to only 3.0 per cent and increased the yield 4.3 bushels an acre with significant odds. Contrary to the above situation the yield increases in most cases following treatment cannot be accounted for on the basis of smut control entirely. The average increases in yield for two years on badly smutted seed treated with dry Uspulun, Tillantin "B," and copper carbonate are 8.1, 7.3, and 5.8 bushels an acre respectively. These increases expressed in percentages are 27.8, 26.8, and 20.9 in the order mentioned above. The average percentage of smut after treatment as well as the percentage of smut reduction due to treatment was nearly the same in each case. In the test in 1927 where 19 replications were made on better soil than in 1926 these variations are greater than an average for the two years. Uspulun reduced the smut 17.2 per cent but increased the yield 31.7 per cent, Tillantin reduced the smut 18.0 per cent and increased the yield by 24.3 per cent, whereas copper carbonate reduced the smut 15.9 per cent and increased the yield only 21.1 per cent. It seems, according to these tests, that Uspulun and Tillantin must have some different effect than copper carbonate, and Uspulun even more effect than Tillantin. One possible explanation is injury which could be more readily charged to the copper carbonate than to either of the other dusts because of the smaller increase in yield. Another possible factor is that other diseases on the seed may be controlled by one dust more effectively than by another.

A species of *Phoma* is commonly found on millet seed in China. This fungus unless controlled could possibly cause some seedling blight. The oospores of *Sclerospora graminicola* also occur commonly on millet seed

from north China. Whatever the cause of these variable results it is evident that the value of a seed disinfectant cannot be measured entirely by its effectiveness in controlling smut. Yield increases or decreases must also be determined.

The effect on yield of the other treatments used in 1927 on artificially smutted seed was not so easily determined. The soil was variable in fertility as evidenced by great differences in the yield of individual check rows not far removed from each other. In addition the untreated seed was not badly smutted. Before any definite conclusions can be drawn it will be necessary to test them for at least one more season. It may be of some significance, however, that only one treatment, namely Tillantin-Nazzbeize, reduced the yield.

COMPARATIVE YIELD OF NEARLY SMUT FREE AND SMUTTED SEED

In order to determine the effect of smut on the yield of grain it is necessary to compare the yields of disease-free and smutted seed from the same source and of the same strain or variety. One method is to inoculate some clean seed artificially and then compare the yield of it with smut-free seed. In any such test the percentage of smut as well as the yield of each sample must be determined. In this experiment another method was used. Fifty smut-free heads were selected at random from a field in which 50 per cent of the heads were partially or entirely infected with smut. These heads were gathered just before harvest in 1925, the seed was threshed and stored separately and used in planting the following spring. From the same field a few pounds of seed were secured after the grain was threshed, at which time all of the seeds were almost black owing to the presence of spores of the millet smut fungus. In the spring of 1926 seed from these two samples were planted in rod rows, which were repeated nine times. Immediately after the rows were harvested, each bundle was labeled and a count made of the total number of heads together with the number of smutted heads. The yield of each row was determined in grams and later computed in terms of bushels an acre. The planting of this test was made a part of a seed treatment study given in table 1. By reference to the table it will be seen that the seed from selected heads had 26.2 per cent less smut than the untreated checks. The checks yielded 26.6 per cent less grain than the seed from selected heads. From this it is evident that in 1926, 26.2 per cent smut reduced the yield by 26.6 per cent. It is worthy of note that although seed from heads selected from a badly infected field had slightly more smut than any treated seed with the exception of the seed treated with Uspulun solution, yet the yield increase was greater than in any of the treatments. Furthermore, this test indicates that most of the smut infection takes place during the threshing operation and for that reason seed

selection before harvest would be of great help in the control of smut, a practice which could easily be carried out by Chinese farmers.

SUMMARY

Tests were carried on at Nanking, China, in 1926 and 1927 with nearly smut-free and badly smutted millet seed.

Formaldehyde, dry Uspulun, copper carbonate, and dry Tillantin "B" were all about equally effective in controlling smut on naturally smutted seed, although none of them reduced the smut to less than 2.6 per cent. The checks showed an average of 26.6 per cent in 1926 and 20.6 in 1927. Uspulun solution gave poor control.

Dry Tillantin "B," Uspulun, Tillantin-Trochenbeize, Tillantin-Nazzbeize, and liquid Uspulun and Tillantin-Hoesht, all reduced smut to less than 1 per cent in comparison with 6.8 per cent smut on untreated, artificially smutted seed.

On badly infected seed, all of the treatments used increased the yield, the increases ranging from 1.7 to 11.7 bushels an acre, depending on the fertility of the soil and the treatment used. Dry Uspulun and Tillantin "B" gave consistently larger increases in yield than copper carbonate.

Smut-free heads selected before harvest from a field badly infected with smut produced a crop with 4.7 per cent smut, which was 26.2 per cent less than that in the crop produced by seed from the same field collected after threshing. The increase in yield from seed of selected heads was 26.6 per cent.

Where a small acreage is planted on each farm as in China, head selection before harvest in combination with seed treatment should prove worth while.

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CONTROL OF BUNT OF WHEAT IN NEBRASKA[1]

GEORGE L. PELTIER[2]

INTRODUCTION

The application of copper carbonate by Darnell-Smith (1) in Australia in 1917, supplemented by the later studies of Mackie and Briggs (6) in California, Heald and Smith (4) in Washington, and others, for the control of stinking smut or bunt of wheat, opened up a new era in the control of this altogether too common and serious disease. The use of copper carbonate dust has rapidly spread over the entire country so that it has now become an established treatment for bunt of wheat. At about this same period there were introduced into this country a number of organic mercury compounds for the control of seed-borne diseases, including bunt in wheat. A number of these dusts, recommended for the control of bunt, were included in the tests.

In the main the many results obtained by the various investigators in the several states have shown a very uniform and marked control, especially with the copper carbonate dusts. Occasionally, however, complaints have been made by growers that the control of bunt has not always been so satisfactory as had been anticipated. This has been especially true in certain sections of Nebraska. In order to determine what factors were responsible for the varying results obtained by farmers in Nebraska, a series of tests was begun in 1923 and continued through 1927 at two places, the Station Farm at Lincoln and the North Platte Substation, located 250 miles west of Lincoln. At North Platte wheat is grown under dry-land conditions, typical of much of the upland of the western part of the state outside of the sand-hill sections.

METHODS

The seed wheat used was Nebraska No. 60, a very uniform strain of Turkey developed by the Department of Agronomy. The original bunt inoculum was obtained through the courtesy of F. N. Briggs. Sufficient inoculum was gathered from the test plats each year for the following year's test, so that probably the same smut strain was carried through the period of the experiments. The same spore load, 5 grams of inoculum to 100

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grams of wheat, was applied to the grain each year. Heald (3) has found that this spore load is necessary to produce maximum smutting. Uniform methods of applying the dust compounds were also employed. All the compounds were from the original containers obtained at the beginning of the tests. During the course of these experiments the following compounds have been used one or more years: copper carbonate, Corona Copper Carb, copper stearate, colloidal copper, Jabonite, Semesan, Bayer dust, Corona 40–S, Corona –620, Seed-o-san (white), Seed-o-san (pink), and formaldehyde. All compounds of unknown composition were discontinued after the first year and are not included in the discussion. All dusts were applied at the rate of 2 ounces a bushel. Formaldehyde was used in the concentration of 1–320, and the seed soaked for 15 minutes. Through the use of the same wheat, same source of inoculum, same spore load, same dust compounds, and the same rate and method of application, the tests were made as uniform as possible.

One-half of each lot of treated seed was retained for planting at Lincoln while the other half was sent to North Platte. The plats varied somewhat in their location from year to year at Lincoln in order to conform with rotations, but were on the same type of soil. This was likewise true of the plats at North Platte, where the seedings were made in connection with other wheat experiments. While the soil varied somewhat in the two localities, the hydrogen-ion reaction was about the same. The only variables in these experiments were the date of planting and the resulting environmental and soil factors encountered by the germinating seeds and growing seedlings in the two localities.

The percentages of smut in the plats given in the tables are based on head counts. For two years counts were made on infected culms also, but no appreciable differences between the two methods of counting were noted. The plats were 1/40th of an acre and were run in either duplicate or triplicate.

RESULTS OF THE TESTS AT NORTH PLATTE AND LINCOLN

At the time the plats were planted, 200 additional seeds of each lot were planted in rod rows, and a count was made in October of the resulting plants and another in April of the number of plants which survived the winter. This was done to determine whether any of the treatments increased or decreased germination and the subsequent survival of the plants. No appreciable differences were noted with any of the treatments with respect to germination, stimulation, and winter survival, with the exception of those treated with formaldehyde, which gave a consistent and lower percentage of germination.

In table 1 are listed the results obtained with the different treatments at North Platte for the years 1923–24, 1924–25, and 1926–27. No bunt was found in any of the plats during the 1925–26 season owing to the low percentages of germination and survival, primarily caused by the dry, windy, and cold season. The average percentage of germination in the rod rows of all treatments in 1925–26 was only 56.5 per cent, while only 15.7 per cent of those which germinated survived, as contrasted with 80.4 per cent germination and 72.1 per cent survival the previous year.

It will be noted that the treatments were effective the first season when only 11.4 per cent of smut was found in the untreated controls. Relatively similar results were obtained in 1926–27, but again only 13.8 per cent of smut was found in the untreated controls. However, in 1924–25, 46.3 per cent of smut occurred in the untreated controls and no treatment was successful in completely controlling bunt; even the formaldehyde-treated seed yielded 6.6 per cent of smut. Some of the treatments, however, greatly reduced the amount of bunt compared with the untreated controls.

The data obtained for three years at Lincoln with the various treatments are also listed in table 1. During the season 1924–25 all dust compounds gave very good results, with the untreated controls showing approximately 15 per cent of bunted heads. During the next two years the untreated controls had a high percentage of infection, approximately 41 and 40 per cent respectively, with considerable bunt showing in most of the treatments

TABLE 1.—*The percentage of bunt in the various tests at the North Platte Substation and at the Station Farm, Lincoln (1923–27)*

Treatments[1]	1923–24	1924–25		1925–26	1926–27	
	North Platte	North Platte	Lincoln	Lincoln	North Platte	Lincoln
	(per cent)	(per cent)	(per cent)	(per cent)	(per cent)	(per cent)
Untreated control	11.4	46.3	14.9	41.2	13.8	39.7
Copper carbonate	0.3	8.6	0.7	7.7	0.08	1.9
Corona Copper Carb	0.4	13.1	1.03	9.1	3.7	5.0
Colloidal copper	—	—	—	23.7	—	—
Copper stearate	—	30.0	1.73	26.8	—	—
Jabonite	—	—	—	—	1.1	11.8
Semesan	0.4	18.7	0.8	11.9	2.2	8.7
Bayer dust	—	—	—	30.3	2.4	18.9
Formaldehyde	—	6.6	0.01	7.0	0.9	2.0
Non-inoculated control	—	2.5	0.06	8.2	0.0	0.6
Date of planting	Sept. 28	Sept. 24	Oct. 16	Oct. 10	Sept. 18	Oct. 9

[1] All seed was inoculated similarly except as noted.

as contrasted with the first year's trials. Even formaldehyde failed to check bunt completely during the season of 1925–26.

INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON BUNT INFECTION

It is clearly evident that although the seed was handled uniformly the effectiveness of the treatments varied not only from year to year in the same locality, but also differed widely during the same season at the two points in the state. The results indicate that when a small to moderate infection occurred in the untreated controls, most of the dust treatments were very effective in controlling smut, whereas when a high percentage of smut was recorded in the untreated controls, these treatments, while giving good control in some instances, were not on the whole nearly so effective. It appears that under conditions favoring smut infection a number of dust compounds for some reason or other are not wholly effective in smut control and this fact may account for some of the complaints received from farmers of the unsatisfactory results obtained with some of these treatments. In order to deduce a possible explanation of these results a brief account of the nature of smut infection and its relation to environmental conditions is presented.

Woolman (9) has found that the fungus enters the epidermis of the coleoptile of both susceptible and resistant wheat plants but that in resistant varieties it penetrates no further. The time required by the fungus to pass into the permanent tissues of the plant he states to be 10 days.

Thomas (8) also reports that the coleoptile is the region of attack, and that after entrance is effected the organism continues to develop within and at the expense of the wheat plant. He also states that infection takes place coincidentally with the germination of the seed and that naturally the period of susceptibility of the seedling is short, as the germinating smut spore can survive for only a limited period in the soil—usually not longer than a month.

According to Percival (7) the coleoptile functions chiefly as a protective cover to the young foliage leaves during their upward growth through the soil and is made of tissues of a simple character. He finds that the coleoptile does not remain active, but gradually shrivels up after the first leaf starts to uncurl.

Judging from the above accounts, infection of the seedling takes place through the coleoptile and the period of susceptibility extends from the inception of germination until the coleoptile becomes functionless. When the extent of the life of the plant is considered, this is an extremely short period. Apparently the environmental conditions do not have any influence on the development of the fungus once the permanent tissues are reached,

so that we are concerned only with the life of the seedling from the inception of germination until the coleoptile shrivels up.

Several experiments were carried out with a number of winter wheats at different controlled soil temperatures to determine first the percentage of germination and second the time required for the seedlings to emerge. All seed was planted at the same depth in a soil with a constant moisture (35 per cent dry weight 62.8 M. H. C.) which was found to be near the optimum. The results of tests are given in table 2.

TABLE 2.—*The influence of various soil temperatures on the percentage of germination and the time required for the emergence of the seedlings of a number of winter wheats*

Soil temperature (degrees C.)	Germination (per cent)	Time for emergence (days)
5	73	22
10	77	12
15	81	8
20	78	4
25	75	3
30	65	2

During these tests it was noted that the hypocotyl was very much elongated at 25° C. and much more so at 30° C. When the containers in which the seeds were planted and held at 30° C. for one week were transferred to 15° C., some seeds germinated, indicating that not all inhibited seeds were killed at the higher temperature.

The striking point brought out by the above data is the fact that there is not much variation in the percentage of germination at the soil temperatures of 5° to 25° C., when the time element is eliminated. The highest percentage of germination was obtained at 15° C., which appears to be near the optimum for germination of the wheat varieties tested. However, when the time for emergence is considered, only 2 days are required at 30° C., while at 5° C. 22 days are required. Even at the optimum soil temperature (15° C.) for germination, 8 days elapse before emergence. Thus the time required for emergence is an important factor, as the infection court, namely the coleoptile, is receptive to infection over a greater period of time at the lower temperatures.

Woolman and Humphrey (11) find that the optimum temperatures for spore germination lie between 18° and 20° C., while the minimum and maximum temperatures respectively are 0° to 1° C. and 25° to 29.1° C. In table 3 the time required for the first evidence of spore germination, as

adapted from the data obtained by Woolman and Humphrey (10 and 11) and others cited by them, is compared with the time necessary for the seedlings to emerge at the same temperatures as shown in table 2.

TABLE 3.—*The time required for seedling emergence at various temperatures compared with the time required for the first sign of spore germination, as adapted from the data of Woolman and Humphrey*

Temperature (degrees C.)	First sign of spore germination (days)	Time for seedling to emerge (days)
5	12	22
10	5	12
15	3–4	8
20	2	4
25	No response?	3
30	No response	2

Thus at the higher temperatures the spores are unable to germinate at all, while at 20° C. and lower the spores germinate in less time than is required for the seedlings to emerge. In all probability the coleoptile develops very slowly at the lower temperature and so provides an excellent infection court for a much more extended period than at the higher temperatures.

Two tests were made to determine the relation of soil moisture to seed germination on several winter wheats. The seeds were all planted at the same depth and a soil temperature of near 20° C. was maintained during the course of the experiment. Soil having a moisture equivalent of 15 was used. Four different soil moistures were tested, as noted in table 4.

TABLE 4.—*The percentage of germination of a number of winter wheats at different soil moistures*

Soil moisture (dry weight in per cent)	Germination (per cent.)
10	68
14	90
18	78
22	79

The optimum soil moisture for germination of the seed tested appears to be at 14 per cent with a moisture equivalent of 15.

Hungerford (5) finds under controlled conditions in the greenhouse "that low soil temperatures and a fairly high percentage of moisture in

the soil are both conducive to stinking smut infection. . . . The highest percentage of infection was secured at temperatures ranging from 9° to 12° C. and in soil containing 22 per cent moisture and with a moisture equivalent of 20.7." He found further "that an exceedingly high percentage of moisture in the soil seemed to inhibit infection." The moisture content of the soil in which he obtained the highest percentage of infection is only slightly higher than that in which the writer found the highest percentage of germination. In this connection Woolman and Humphrey (11) state that the germination of the spores in a saturated soil is inhibited, the spores losing their viability in about 35 days. They regard the optimum soil moisture content for infection in the basaltic soil of the Palouse country to be between 16 and 30 per cent. Therefore it seems probable that the soil moisture best suited for the germination of the seed is the one most conducive to spore germination and subsequent infection. Extremely low and high soil moistures seem to inhibit the germination of the spores to a greater extent than they do the wheat seed. On the whole it appears that soil moisture is not so important a factor in smut infection as soil temperature.

Hungerford (5), Woolman and Humphrey (11), Faris (2), and others have shown that soil temperatures below 20° C. are very conducive to bunt infection. Hungerford obtained the highest percentage of infection under control conditions at temperatures from 9° to 12° C. Likewise, Faris reports maximum infection at temperatures of 5°, 10°, and 15° C. As pointed out previously, spore germination decreases very rapidly above 20° C., which may account for the small amount of bunt reported by these workers at temperatures above this point. From these studies it is reasonable to conclude that soil temperature is the most important factor involved in bunt infection in the field, providing smutty seed is planted.

Unfortunately soil temperatures were not recorded at Lincoln and North Platte during the years the tests were made. However, on comparing the air temperatures for these years it is found that general analogies can be drawn from these data. During the fall seasons of 1923 and 1926 at the North Platte Station the rather low percentage of infection in the untreated plats can be correlated with temperatures higher than those occurring in 1924, when the percentage of bunt in the untreated controls was almost four times as large. The entire fall and winter season of 1925 was so unfavorable to the growth of the wheat itself that no bunt developed in any of the plats. At Lincoln rather higher temperatures prevailed during the infection period in 1924 than 1925 and 1926, the percentage of bunt in the untreated control plats being three times as large the last two years.

The mean temperatures during the infection period in 1924 at North Platte were much lower than those recorded at Lincoln, which accounts for

the high percentage of infection (46.3) in the untreated control plats at North Platte and the rather low percentage of infection (14.9) at Lincoln, although the same inoculated and treated seed was used in both localities.

The data show that when the incidence of bunt infection is low, most of the seed treatments tested are more or less effective; whereas when the percentage of infection is high, the less effective treatments are easily recognized. Under these conditions the effectiveness of all treatments is lowered. This was true even with formaldehyde. The only explanation that can be offered is that, when environmental conditions favor maximum bunt infection, the dust treatments are not completely effective in preventing some of the bunt spores from germinating and entering the plant tissues. In testing dust treatments for the prevention of bunt, they should be carried out not only in different localities but also under conditions which will produce maximum infection. In this way the less effective treatments can be more easily eliminated. The wheat grower should be urged to plant treated seed, as nearly at the normal planting date for his locality as possible, to avoid conditions favoring bunt infection.

SUMMARY

1. No appreciable differences were noted with any of the dust treatments with respect to germination, stimulation, and winter survival, except in the tests with formaldehyde, which gave a consistent and lower percentage of germination.

2. Formaldehyde was the most effective treatment under all conditions. Of the dust treatments, applied at the rate of 2 ounces a bushel, copper carbonate gave the most effective control. Corona Copper Carb was slightly less effective.

3. None of the other copper or organic mercury compounds showed as great a degree of control as the copper carbonates, especially under conditions favorable for maximum bunt infection.

4. It appears that the effectiveness of all treatments tested is lessened in varying degrees under environmental conditions favoring maximum bunt infection.

5. Treated seed should be sown as nearly at the normal date of planting for the locality as possible.

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ENDOSEPSIS AND ITS CONTROL IN CAPRIFIGS

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In recent years the calimyrna[1] fig industry in California has been seriously threatened by the increasing prevalence of three diseases of the fruit: smut (7), souring (3), and endosepsis (1), which during the past three years have caused the growers an estimated annual loss of well over 50 per cent of their crop. In addition to this loss of crop the hand-sorting of both fresh and dried fruits which has become necessary because of these diseases has so raised the cost of production that the industry in some districts has ceased to be a profitable one.

Of the three diseases named above, endosepsis is by far the most important, being easily responsible for 80 per cent of the cull pile. This disease was formerly recognized under the various names of "brown rot," "pink rot," "soft rot," and "eye-end rot," terms that are strikingly descriptive of various phases and symptoms of the disease but somewhat confusing because of their multiplicity and quite undesirable because of their use in connection with specific diseases of other fruits. It is now known under the name of "endosepsis," a Greek term which means internal rot. Caldis, who described and named the disease (1), has worked out its transmission and etiology and named the causal organism *Fusarium moniliforme* var. *fici* Cald.

This fungus grows in the cavities of caprifigs where it sporulates abundantly. When the caprifying insects, *Blastophaga psenes* L., emerge from the galls they pick up spores on wings and other body parts and carry them to other caprifigs or to the calimyrnas, where they germinate and produce the endosepsis.

We have no authentic report of endosepsis occurring in calimyrnas prior to 1920 (8). Since then the disease has been found in every fig-growing section in California, with losses estimated at from 20 to 90 per cent (1).

Since it has been shown that the internal rot is transmitted from the caprifigs to the calimyrnas by the caprifying insect, it seems logical to assume that if the causal organism were eliminated from the caprifigs there would be no spores for the insects to pick up and the rot in the calimyrnas would be automatically controlled. This paper, as the title indicates, deals mainly with the various manifestations of the causal organism in caprifigs and its eventual elimination from them.

[1] The calimyrna is the Lob Injir variety of Smyrna grown in California.

INTERRELATION OF FIG, FUNGUS, AND INSECT

Technically the insect *Blastophaga psenes* L. is considered to be parasitic on the caprifig, but actually the relation is a truly symbiotic one, as neither can properly develop, mature, or propagate without the other. The relation between the fungus and the caprifig is one that might be called inactively parasitic on the part of the fungus and rather passive on the part of the host. The fungus does not invade actively growing or healthy tissues but subsists on non-living material such as floral and other parts of the fig that have become inactivated through age or been killed by ovipositing blastophaga or other agents, and on the dead bodies of the insects themselves. The tissues of the fig are, therefore, not extensively invaded until the fruit is ripe; hence it develops and matures in normal manner and season with no apparent ill effect due to the presence of the fungus. From the standpoint of the fungus the triple alliance (fig. fungus, and insect) is a most favorable one, especially to propagation and distribution; the comparatively dry meat of the fig, though unfavorable to much vegetative growth, is an excellent medium for the production of spores, and the large numbers of insects present at the time of maximum sporulation furnish a convenient means of ready transportation.

METHODS OF DISSEMINATION

Climate or temperature is the most important factor in the maturing of caprifigs, much more so than in the calimyrnas. Hence in sections where caprifigs mature late the grower frequently imports profichi from districts where caprifigs mature early in order to get the first part of his calimyrna crop properly caprified, and vice versa. Likewise after winters when local frost has destroyed the hold-over capris, reestablishment of the blastophaga is brought about by importation of mamme figs from sections where the frosts were less severe.

Each caprifig contains an average of four hundred insects (4). If infection had taken place in only one mamme fig it would be theoretically possible, assuming that each insect went to a different fig, to have as many as four hundred infected figs in the following crop (profichi) and in the succeeding crop (mammoni) as many as one hundred and sixty thousand (160,000). In nature this phenomenal increase from 1 to 160,000 in one year does not occur for the following reasons: (*1*) Usually several insects enter the same fig, thus decreasing the number of figs entered and infected; (*2*) The bulk of the profichi crop being used for pollinating the calimyrnas is removed from the capri trees, and thus, though they cause rot in the edible figs, they are prevented from causing further increase in the number of caprifigs infected. (*3*) Not all insects emerging from an infected fig

carry the fungous spores for the reason that sporulation sometimes, in certain varieties and under certain conditions, does not begin until one or two days after the insects have begun to issue. If, however, such figs have frost lesions or other injuries that give rise to dead tissues in the fig, then sporulation will be abundant before emergence and practically all the insects issuing will become infected. (*4*) Not all insects find their way into figs. The practice of intersectional distribution of caprifigs, the ability of the insects to fly considerable distances combined with the rapid natural increase in the disease once infection has taken place appear to offer an adequate explanation of the almost epidemic rapidity with which endosepsis has increased and spread.

CONTROL OF ENDOSEPSIS IN CAPRIFIGS

In view of the fact that the structure of the fig does not allow ready application of fungicides, it seemed that the only way to effect control of endosepsis would be to find non-infected caprifigs, propagate them in isolated places where all other caprifigs had been removed and in this manner establish a colony of blastophaga free from the fungus. Several difficulties attended this method of procedure. In the first place clean figs would have to be sought for by a hit-and-miss method as there are no external symptoms of the disease in caprifigs, and with the high percentage of infection clean figs were seldom found. In the second place, the only way definitely to determine the presence or absence of the fungus in a fig is to culture it. During the process of culturing the insects are killed and therefore valueless for propagation. For several years Dr. P. D. Caldis and others tested caprifigs from various parts of the state in an effort to find and establish a clean colony of blastophaga. Though a few individual figs were found that apparently did not carry the fungus, no colony of clean blastophaga was successfully established.

When the writer commenced work on control of endosepsis in July, 1926, it occurred to him that by means of a hypodermic syringe it might be possible to inject into the cavities of caprifigs a fungicide that would kill the fungus without injuring either the fig or the insects still in the galls. The preliminary experiments carried on with mammoni figs gave very promising results (6), showing that by the injection method clean figs and insects could be produced. The injection method, however, had some very serious limitations that made it undesirable for use on a large scale. The operation requires considerable skill and very great care, making it a slow and tedious affair. Because of internal structure (solid center) most varieties of caprifigs can not be treated by this method. To be effective, injection must be done over a short period of time, preferably within six or eight weeks after the figs have been caprified. And finally, when inject-

ing a large number of figs on a tree, a few might easily be overlooked and left untreated to start infection over again. Obviously a method that would overcome these difficulties would be desirable. As has been pointed out before, in the mature caprifig the fungus is too deeply imbedded in the tissues of the host to be reached and killed by a fungicide, but it was thought that sterilization of the fig cavity and gall surfaces would not only kill all superficial mycelium and spores but would also delay further sporulation until most of the insects had emerged. The following experiment was devised to test this assumption. Ninety mature mamme figs were selected from which the insects were either actively emerging or just about to emerge. These figs were cut into halves. One half of each was treated by immersion in 2 per cent Semesan for 15 minutes and the other half was kept as an untreated control. The halves, both treated and untreated, were placed separately in sterile bottles for observation and examination. In addition 20 whole untreated mamme figs were placed in sterile bottles to see if cutting into halves would affect the percentage of infected insects. As the insects emerged, about 10 from each bottle were placed on nutrient culture media each day for seven days to determine their sterility. The results are shown graphically in figure 1.

The two upper curves (1 and 2) in figure 1 show a considerable difference in infection of insects emerging from whole and cut figs. The reason is probably this: that all insects leaving a whole fig must pass through the eye, which, in the mamme, as well as in all other caprified figs, is the principal place of infection because the entering blastophagas usually tear off their wings and leave them wedged between the scales surrounding the eye of the fig. These wings, bearing numerous setae, are almost ideal mechanisms for carrying spores of various kinds.

The two lower curves (3 and 4) show that in the dipped figs fully 90 per cent of the insects emerge before the fungus has developed enough to infect them. They also appear to show by their overlapping that figs so treated must be destroyed within five days to obviate reinfection from insects emerging later. Under the conditions of the experiment both temperature and humidity were higher and more constant than in the field. Since these conditions hasten the emergence of the blastophaga and also the development and sporulation of the fungus, we would under field conditions expect curves 3 and 4 to be altered somewhat in shape but very little in their relation to one another. In general, results seem to indicate that the objectionable features attending injection can be eliminated by use of the cutting and dipping method.

On the basis of results obtained in the preliminary experiments it was decided to test the two methods on a larger scale in the field. Caprifig

groves were selected at various places in the state on the basis of their isolation from other capri trees, to minimize the danger of insects flying in from infected caprifigs. The groves were located as follows: Fresno, Calif. (12

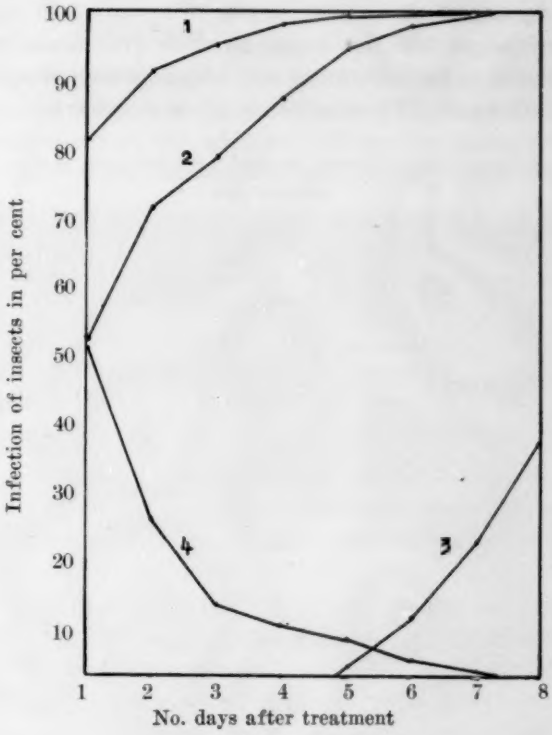

FIG. 1. The relation of rate of emergence to rate and amount of endosepsis infection of *Blastophaga* from treated and untreated caprifigs.

Curve 2. Percentages of infected insects from 20 whole, untreated mamme figs.
" 2. Percentages of infected insects from 90 halves of untreated figs.
" 3. Percentages of infected insects from 90 halves of treated figs.
" 4. Rate of emergence of insects. This appeared to be practically the same for treated and untreated figs.

large capri trees); Merced, Calif. (10 medium-sized capri trees); Madera, Calif. (30 large capri trees). At these places all the hold-over figs (mamme) were removed and destroyed. At a fourth place located at Davis, Calif. (12 capri trees), no treatments were given and this place was chosen as a control. At two other places near Fresno several thousand mamme figs were injected with 2 per cent Semesan during January, 1927. When these figs became mature they were picked and used to effect recolonization at

Fresno and Merced. For recolonization of the profichi at Madera the dipping method was employed. Mature mamme figs were cut, dipped in 2 per cent Semesan for 15 minutes, and then hung in the trees in small baskets. At intervals of four days these figs were removed and burned and replaced by others.

As soon as the profichi figs began to ripen representative numbers of them were brought to the laboratory and tested by microscopic and cultural methods for endosepsis. The results are given in table 1.

TABLE 1.—*Results from culturing profichi caprified with insects from treated mamme figs*

Place (California)	Method of treatment	No. figs cultured	No. figs infected	Percentage infected
Fresno	Injection	400	4	1.0
Merced	Injection	178	0	0.0
Madera	Dipping	208	0	0.0
Davis	None	100	100	100.0

The data in table 1 indicate that *Fusarium moniliforme* var. *fici* Cald. may be eliminated from caprifigs either by injecting Semesan into green ones or by cutting mature ones into halves and immersing them in the fungicide.

Though the cutting and dipping method appears to be quite effective, it has two drawbacks that would seriously hamper its application on a large scale. (*1*) the figs must be left on the trees until they are mature so as to enable the bulk of the insects to emerge within a few days after disinfection. Under this condition many insects escape prior to the treatment, making thorough control impossible. If the figs are picked several days before maturity, the insects, unless artificial heat is applied, develop at so slow a rate that only about 20 per cent of them emerge during the four or five days when sterilization is effective. (*2*) The dipped figs must be destroyed within five days of treatment.

To obviate these drawbacks a new plan was devised (9). It has previously been thought that the blastophaga insect is very short-lived after it emerges from the gall (4). The writer found by experiments that emerged insects placed in glass vials or other containers and stored in the dark at temperatures ranging from 35° to 50° F. would remain alive for eight days and at the end of that time were able to enter and properly fertilize caprifigs. The longevity of the insects, together with the facts that they are positively phototropic and in their rate of emergence respond readily to heat, made it possible to augment the dipping method by insectary practices.

Mamme figs were picked about four weeks before maturity and stored at about 60° F. They were then treated by the dipping method and placed in a specially constructed incubator (Fig. 2) at about 85° F. At this temperature it was found that from 60 to 80 per cent of the insects would emerge within 24 hours. The insects were made to emerge into sterilized vials. When about 500 insects had entered a vial it was removed from the incubator, corked, and cooled to about 45° F. Shipments of such vials by mail and express to various parts of the state indicated that, if properly handled, the insects were not adversely affected by transportation, as upon liberation in capri trees they entered and properly fertilized the caprifigs.

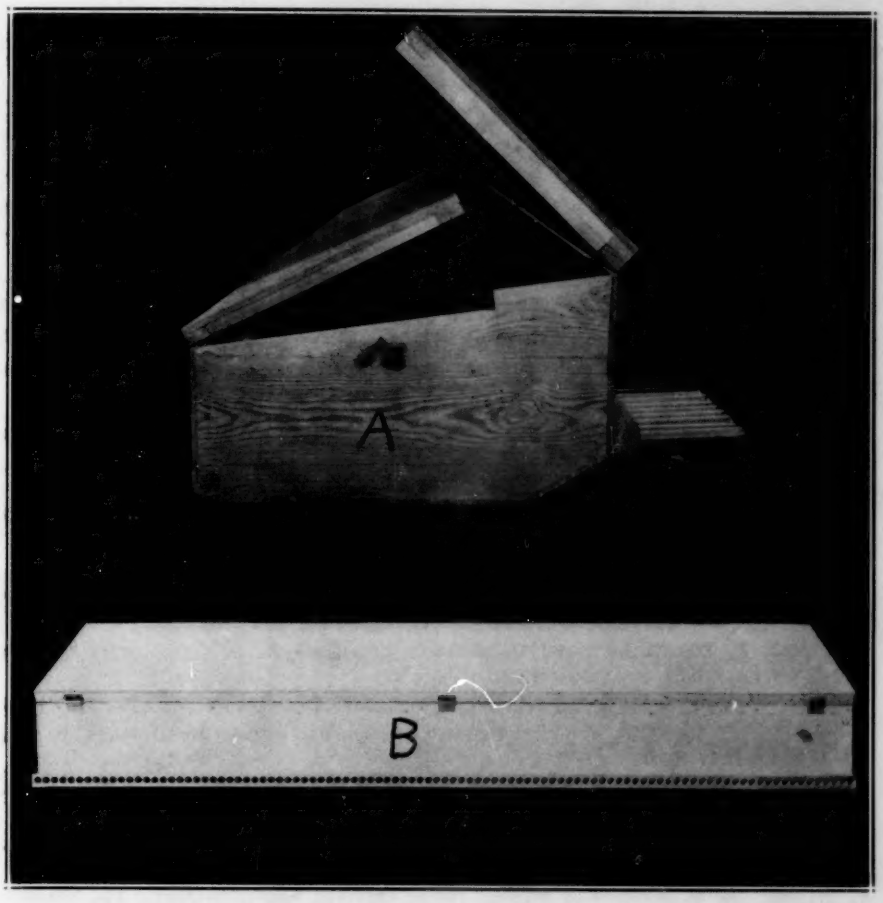

FIG. 2. Special incubator. A. End view, with glass vials in place. B. Front view. The only light entering the incubator comes through the holes in which the glass vials are placed. The dimensions of the incubator are approximately 1 ft. x 3 ft. x 10 ft. The bottom is of sheet metal; the front, rear and top of celotex; and the ends of wood.

By this plan it should be possible to remove all the mamme figs in the state and ship them to an insectary in a place where no blastophaga could escape to any caprifig trees. There the figs would be stored and treated by the dipping method. By use of special equipment and by application of heat the insects at the proper time would be made to emerge into sterile vials in which they might be shipped to any part of the state and placed in trees to caprify the profichi crop. This method would make it unnecessary to remove any treated figs from the trees, furnish large numbers of clean blastophaga, and make it possible to clean up the whole territory in one season.

SUMMARY

1. A rot (endosepsis) of the fruit of caprified figs in California has assumed alarming proportions in recent years.

2. The causal organism, *Fusarium moniliforme* Sheld. var. *fici* Cald. grows and develops in the various crops of caprifigs and is transmitted from them to the calimyrnas by the caprifying insect *Blastophaga psenes* L.

3. The rapid increase and spread of the disease is attributed to the practice of inter-sectional distribution of caprifigs, to the prolific nature of the insect and its ability to fly long distances.

4. Laboratory and field experiments indicate that the causal organism may be eliminated from caprifigs either by injecting a fungicide (2 per cent Semesan) into young capri (mamme) or by cutting mature ones into halves and dipping them for 15 minutes in the fungicide.

5. The dipping method may be made adaptable to large-scale use by application of insectary methods.

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DEVELOPMENT OF STORAGE DISEASES IN SUGAR BEETS RESULTING FROM HOOK INJURY

C. M. TOMPKINS AND S. B. NUCKOLS[1]

The sugar beet (*Beta vulgaris* L.) is especially susceptible to fungous invasion immediately following any form of bruising or mechanical injury at harvest time, because of the comparatively thin layer of cork cells constituting the periderm. Injuries to the root may occur in a number of ways, but this paper is concerned particularly with wounds in the beet occasioned by the use of the so-called hooked beet knife, with subsequent development of decayed areas due to fungous attack.

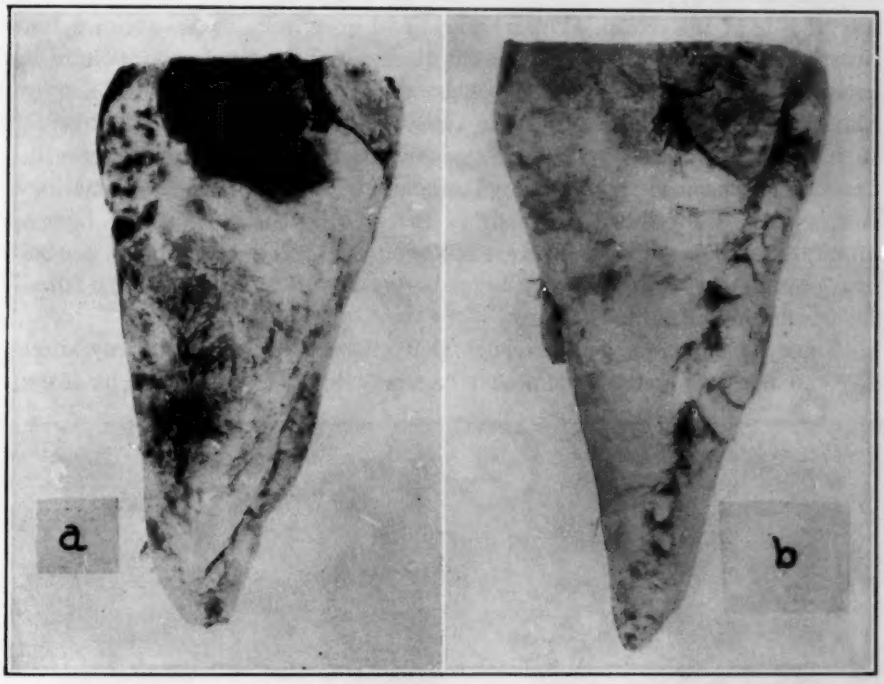

FIG. 1. Sugar-beet roots, (a) showing hook injury and splitting of tissues with subsequent development of decay; (b) showing hook injury and subsequent development of decay.

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In the United States, topping machinery has not come into commercial use and practically all sugar beets are topped by hand. A straight beet knife is used in Colorado, Nebraska, Montana, Michigan, and Ohio, the use of which necessitates raising each individual beet by hand from the ground or nearby pile. This method precludes the possibility of injury to the beet root, insofar as topping practice is concerned, and is to be highly recommended. In the states on the Pacific slope, including Utah, Idaho, Washington, and California, a hooked knife is used. This knife is approximately the same size as the straight beet knife but in addition is equipped with a sharp-pointed, curved hook at the extremity of the knife blade. The hook is 7 cm. long, with a mean diameter of 7 mm., and tapers down to 2 mm. at the tip. The tapered portion of the hook occupies a length of not more than 10 mm. In order to raise a beet from the ground, the laborer, with a quick stroke of the knife, drives the hook into the root, usually on the lateral side of the crown. Observations and examinations have shown that invariably the hook is forced into the tissues sufficiently far to include its greatest diameter. When the hook is withdrawn, a wound with a mean diameter of 7 mm. has been made (Fig. 1). Often this wound is larger than 7 mm., particularly if difficulty is experienced by the laborer in withdrawing the hook from the beet when once it has been raised. Sometimes two or three wounds will be made in the root by the hook as the laborer attempts to hook the beet. Severe splitting of the tissues is not an uncommon occurrence. Often a very ragged edge results which is ideally suited for incubation of fungous spores.

Since factory capacities are such that all beets harvested in any single day can not be processed immediately, many tons of roots must be stored

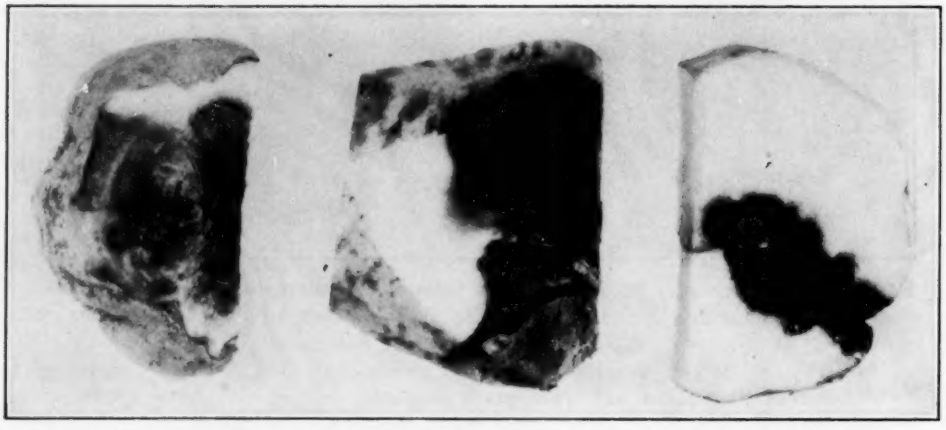

FIG. 2. Cross and longitudinal sections through the center of the injured portions of diseased sugar beets, showing the effect of hooked-knife injury.

for various periods, the average of which is approximately 40 days. Parasitic fungi, under favorable conditions of air temperature and humidity, are able to cause decay in wounded beets to the extent of from 15 to 25 per cent of the total weight of any individual root in a relatively short time. Frequently one-quarter to one-half of the root will become involved (Fig. 2). In commercial storage piles where beets are held for from 25 to 40 days or longer, it is estimated that a loss of not less than 15 per cent is sustained. This includes both weight and sugar loss. Root injury such as described above constitutes a source of tremendous but avoidable loss to sugar companies.

Numerous isolations have been made from diseased beets, disclosing the fact that *Phoma betae* is the principal wound parasite responsible for decomposition of the tissues. Several species of *Fusarium* also are parasitic. These organisms are unable, apparently, to parasitize the beet root in the absence of wounds.

In order to prevent future losses in storage from this source, it is recommended that the use of the hooked knife be dispensed with and the Colorado system of harvesting substituted.

OFFICE OF SUGAR PLANTS, BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
IN COOPERATION WITH
UTAH AGRICULTURAL EXPERIMENT STATION,
LOGAN, UTAH.

PHYTOPATHOLOGICAL NOTES

Observations on the discharge of ascospores of Venturia inaequalis in Maryland.—Prior to 1925 it was customary for Maryland fruit growers to make their first spray application for the control of apple scab during the pink-bud stage of the tree. The failure of many of these growers to control scab successfully led to studies on the time of first ascospore discharge in the spring. The results of these studies during the past four years are as follows.

In the spring of 1925 fallen apple leaves containing scab lesions were collected in an orchard in the southeastern part of Maryland. On March 2 they were placed in moist chambers, one lot being kept in a greenhouse and another lot in an insectary outside of the building. The leaves were moistened at intervals of one to two days. The leaves in each chamber were covered with a piece of large-mesh wire screen, like that used on basement windows. Microscope slides were greased with vaseline and placed on the wire screen over the leaves. These slides were replaced daily and examined with a microscope. On March 27 the first ascospores were found on slides from the greenhouse, and on March 28 the first spores were found on the slides from the insectary. The average temperature in the greenhouse during the period March 2 to 27 was approximately 60° F., and that in the insectary during the same period was about 45° F. For several days after March 28 ascospores were found on both sets of slides. It was thus evident that ascospore development was practically as rapid at an average temperature of 45° as it was at 60° F. from March 2 to 27.

In the spring of 1926, fallen apple leaves containing scab lesions were collected in five orchards located in different parts of the state. These leaves were placed on burlap sacks and held in place with large-mesh wire screening, which was spiked to the ground. Two lots were placed in each orchard. One lot was left under natural conditions and the other sprinkled daily with tap water. Slides greased with vaseline were placed over the leaves and examined daily. The first dates when ascospores were found are indicated in table 1.

In all cases there were several showers and rains between the time observations were begun and the time the first ascospores were found on the slides. At Ridgely there were several more showers than in the other orchards where the work was conducted. In no cases did the number of rains equal the number of times the leaves were moistened. In all orchards, except at Ridgely, there were several rains between the time of finding the first ascospores on the slides over the moistened leaves and the finding of the

TABLE 1.—*The maturation of ascospores of Venturia inaequalis in Maryland in the spring of 1926*

Place	Date of collection	Date on which ascospores were first found[a]	
		Over moistened leaves	Over leaves under natural conditions
Ridgely	March 19	April 28	April 29
Snow Hill	March 24	April 15	May 3
Whiteford	April 4	April 21	May 15
College Park	March 28	April 23	May 16
Newburg	April 3	April 12	April 18

[a] Ascospores caught on glass slides exposed above the infected apple leaves.

first ascospores on slides over the leaves kept under natural conditions, indicating that ascospores on the latter were not ready to discharge so soon as those on the moistened leaves. In all cases there was rain on the day the first ascospores were found on the slides over leaves kept under natural conditions. It thus appears that up to a certain point the more frequently the apple leaves are moistened the earlier the ascospores will mature.

The season of 1926 was unfavorable for the development of apple scab in Maryland, and scab lesions were found on only a very small number of apples leaves. When the studies of the perithecia were resumed in the spring of 1927, it was almost impossible to find any fallen leaves showing scab lesions. However, fruiting bodies which could not be distinguished from the perithecia of *Venturia inaequalis* (Cke.) Wint. were found on practically every fallen leaf which was examined. Many of the leaves were practically covered with them, and they were apparently as prevalent on leaves from sprayed orchards as they were on leaves from unsprayed orchards. When these bodies matured they developed asci and ascospores that were identical in measurements and appearance with those developed in perithecia on leaves with conspicuous scab lesions. In the spring of 1928 the perithecia of *V. inaequalis* (Cke.) Wint. were again found on practically all of the fallen leaves examined from sprayed and unsprayed orchards in all parts of Maryland. No visible scab lesions could be found on most of these leaves, although they bore abundant perithecia.

Perithecia were thickly scattered over the entire surface of some of the leaves, including the midrib and the petiole, while on others they were found only in scattered groups. Perithecia occurred on either the upper or lower sides of the leaves, being most abundant on the surfaces exposed to light. They were found abundantly on all varieties examined, including Winesap, Stayman Winesap, Northwestern Greening, Yellow Transparent, Delicious, Ben Davis, and York Imperial.

In 1927 the first ascospore discharge occurred at widely different stages in the development of the trees in different sections of the state, varying from the so-called "pre-pink" stage in the southeast to the calyx stage in the extreme western part. In 1928, in the extreme southeastern part of the state, the first discharge occurred while the trees were still dormant.—R. A. JEHLE and H. A. HUNTER, University of Maryland, College Park, Md.

Bacterial red-stripe disease of sugar cane in countries of the Pacific.—Bacterial red-stripe disease of sugar cane was first recorded in 1924 from the Hawaiian Islands,[1,2] where it was of economic importance only upon Tip varieties of cane, which are gradually being supplanted by higher-yielding varieties.

There is a bacterial red-streak disease of sugar cane in Queensland, Australia, reported in 1926 by W. Cottrell-Dormer,[3] which in photographs appears identical with the bacterial red-stripe disease found in Hawaii.

Edgerton and Christopher[4] recorded the presence of the same disease in Louisiana three years later on the variety D. 74, a variety which also is gradually being supplanted by higher-yielding canes.

In a recent report by Bolle,[5] red-stripe disease of sugar cane has been recorded from Java. The cane affected was the Java variety, P.O.J. 2722.

In the Philippines, specimens of a disease were collected by one of the writers of this note (Pierce), and on the arrival of Lee in the Philippines it was identified as identical with the red-stripe disease of sugar cane previously recorded in the Hawaiian Islands. The cane affected was the Java variety, P.O.J. 2883.

These findings point to the rather general distribution of the disease in countries of the Pacific, although its presence is not recognized until susceptible varieties are grown. The disease is of very little commercial importance at the present time, because most of the standard sugar cane varieties are highly resistant to it.—H. ATHERTON LEE and W. DWIGHT PIERCE.

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[2] LEE, H. ATHERTON, J. P. MARTIN, HELEN A. PURDY, CLYDE C. BARNUM, and D. M. WELLER. Red-stripe disease studies. Hawaiian Sugar Planters' Assoc., Experiment Station Publication. September, 1925.

[3] COTTRELL-DORMER, W. Notes and observations on the red streak associated with Queensland top-rot disease. Queenland Agr. Jour. 25: 406–414. 1926.

[4] EDGERTON, C. W., and W. H. CHRISTOPHER. Red-stripe disease of sugar cane. The Planter and Sugar Manufacturer, 79: 63. 1927.

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Cladosporium Diseases.—A recent article[1] by Bensaude and Keitt, "Comparative studies of certain Cladosporium diseases of stone fruits," interested me somewhat because many years ago I published a good many notes on *Cladosporium* on several of our stone fruits. Bensaude and Keitt evidently overlooked these early papers.

I first came in contact with the fungus on plum and cherry in 1889. Somewhat later I discovered a scab on the American plum, which I thought might be caused by a new species of *Cladosporium*; but, after a careful study of the mycelium and spores, I came to the conclusion that the fungus was identical with the one on peach. The *Cladosporium* on peach had been studied by Erwin F. Smith.[2]

In the Ottawa Naturalist for November, 1892, is an article entitled "A destructive disease affecting native plums," written by John Craig. Most of the article was written by myself and should have been in quotation marks.

I made numerous references to the *Cladosporium* on stone fruits in my notes on diseases of plants at Ames, published in the Proceedings of the Iowa Academy of Science **2**: 207. 1894. I also mentioned it in a paper on "New fungous diseases of Iowa," published in the Journal of Mycology, **7**: 95–103. 1891. In that paper I referred to its having been seen previously by H. Osborn and to the careful work of Van Thümen, Erwin F. Smith, and B. T. Galloway. Those specimens from Ames were submitted to J. B. Ellis, who thought that the fungus might be a distinct species, but after careful study I concluded that the fungus was *Cladosporium carpophilum*.

I have referred to this *Cladosporium* on stone fruits and the importance of the disease to horticulture in many subsequent papers (See reports of Iowa State Horticultural Society, Iowa Academy of Science, Society for Promotion of Agricultural Science, Iowa Agricultural Experiment Station, and Iowa Weather and Crop Service).—L. H. PAMMEL, Iowa State College of Agriculture, Ames, Iowa.

[1] Bensaude, M., and G. W. Keitt. Comparative studies of certain Cladosporium diseases of stone fruits. Phytopath. **18**: 313–330. 1928.

[2] Smith, Erwin F. Spotting of peaches. Jour. Mycol. **5**: 32–33. 1889.

REPORT OF THE TWELFTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The meetings were held in conjunction with those of the Pacific Division of the American Association for the Advancement of Science and Affiliated Societies at Pomona College, Claremont, California, June 13–16, 1928, and were called to order by vice-president A. G. Milbrath.

A business meeting was held and the following officers elected to serve the society the next two years:

President ..... J. W. HOTSON, University of Washington, Seattle.
Vice-President ..... EUBANKS CARSNER, University of California, Citrus Experiment Station, Riverside.
Secretary-Treasurer ..... B. A. RUDOLPH, University of California, Deciduous Fruit Station, San Jose.
Councilor ..... J. T. BARRETT, University of California, Berkeley.

A motion was introduced to appoint a committee to draw up a suitable amendment to the constitution which would make it possible for the Society by vote of its members present at annual meetings to express to the author of the most outstanding piece of research presented at the meetings its admiration and approval in a material way in the form of a book on plant pathology or kindred subject, a bit of apparatus, or even a sum of money. The motion lost by one vote.

A suggestion that the Society undertake the preparation of a highly illustrated manual of plant diseases found in the territory embraced by the Pacific Division and suitable as a ready reference book for field use rather than for technical laboratory work met with general favor. Owing to the lateness of the hour no action was taken.

A motion to appoint a committee to draw up an amendment to the constitution providing for the automatic elimination of members not in financial good standing in the Society was passed. This amendment must be submitted to the members of the Society and accepted by a majority vote before it can be introduced into the constitution.

Twenty papers were presented, the titles and abstracts of which follow.

B. A. RUDOLPH, *Secretary-Treasurer.*

ABSTRACTS

Rusts of the Pacific Northwest.—J. W. HOTSON.

The article consists of a somewhat elaborate key to all the rusts reported from Washington, Oregon, Idaho, and Montana. Since individual rusts in general are restricted quite closely to a few hosts, an attempt has been made to make a key on the basis of these hosts. All the rusts of the region are divided according to the host-family, and then subdivided under the genus of the host. The individual characteristics of the rusts are used to separate those occurring on the same genus.

Armillaria mellea in mines and wells.—J. W. HOTSON.

Armillaria mellea has been found abundantly on wooden supports in several coal mines in Washington. It has also been found on wooden curbings in wells. In both locations rhizomorphs were abundantly produced, hanging down in great festoons from the timbers in the mines, and floating on the water in the wells. Although the cultures obtained directly from a well have not as yet produced sporophores, the form and structure of the rhizomorphs produced are identical with similar cultures made from the spores of *Armillaria mellea*, leaving little doubt as to the identity of the fungus.

An Aspergillus attacking mealybugs in insectaries in southern California.—A. M. BOYCE and H. S. FAWCETT.

A species of *Aspergillus* appears to be a potential post in insectaries where mealybugs are propagated for their value in rearing beneficial insects. Notes are given on the culture and parasitism of this fungus.

Inheritance of resistance to blast in oats.—W. W. MACKIE.

Blast in oats causes sterile, blanched, and reduced spikelets, resulting in severe crop losses. Many attempts to isolate a causative organism have failed. Explanations for its cause on the basis of malnutrition have not satisfactorily accounted for the disease. Many years of observations with over 300 varieties of oats indicate that varietal resistance is approximately stable. A cross between Kanota, an approximately immune variety, and Richland, a susceptible variety, gave all susceptible plants in the F_1 generation. In the F_2 generation the plants were observed to resist blast as follows:

Plants highly susceptible	108
Plants moderately susceptible	356
Plants immune or with only a trace	178

These numbers indicate a very close approximation to a 1:2:1 Mendelian ratio, indicating a single factor for blast resistance.

Phytophthora in relation to crown rot of walnut.—J. T. BARRETT.

The disease of English walnut trees known as crown rot has been under observation about five years. The lesions are at first confined to the crown and to the basal part of the roots of the Cailfornia black walnut species used as stocks. The southern black species, *Juglans californica*, seems more susceptible than the northern species, *J. hindsii*. The white or English root is very resistant. The crown rot lesions may extend onto the English walnut trunk following girdling of the crown and a weakened condition of the tree. It is from these trunk lesions that *Phytophthora cactorum* has been repeatedly

isolated. The disease has been produced by inoculation with this organism into both the black and English species.

The inorganic nutrition of the fungi. I. *The relation of calcium and boron to growth and spore formation.*—A. R. DAVIS, R. H. MARLOTH, and C. J. BISHOP.

Information on the inorganic salt requirements of the fungi is meager, particularly so with respect to elements which may be needed in small amounts. C. P. chemicals employed in making up nutrient media usually contain as contaminations sufficient amounts of many elements to supply the needs of the organism. In the present experiment the salts were carefully recrystallized and purified. In the case of *Aspergillus niger* and *Penicillium italicum* 50 cultures were grown on media made up of purified and unpurified salts with and without calcium, and the data treated statistically. With both these organisms calcium was shown definitely to increase yield over that obtained in media devoid of this element, and spore formation was dependent upon the presence of the ion. When *Dothiorella sp.* was employed as a test organism, the absence of boron in the medium decreased yields about one half, and the addition of one and one half part to a million restored yield to 90 per cent of that obtained with the unpurified salt. The evidence indicates that both calcium and boron are to be regarded as essential and not as stimulants in the older sense.

The effect of mixed inoculations of certain citrus fruit-rotting organisms.—G. SAVASTANO and H. S. FAWCETT.

Inoculations were made in uniform wounds of orange and lemon fruits with mixed suspensions of spores from pure cultures of different species of fungi and the effect of these mixtures compared with that of each fungus inoculated singly. Mixtures of two or more of the following Citrus-fruit-rotting fungi were used: *Penicillium italicum, P. digitatum, Aspergillus niger, Trichoderma lignorum, Oospora citri-aurantii, Pythiacystis citrophthora, Botrytis cinerea, Sclerotinia libertiana, Alternaria citri, Phomopsis californica, Diplodia natalensis,* and *Dothiorella ribis.* Different lots of fruit were placed at maintained temperatures from 3° to 33° C. Some of the most important features brought out by this investigation were (*1*) the selective effect of temperature in enabling one organism in a mixture to dominate over the others in producing decay; (*2*) the depressing or accelerating effect of given mixtures on the rate of decay as compared with that produced by the most rapidly advancing organism of the mixture when used alone; (*3*) the influence of given mixtures of organisms on the type of decay, its color and consistency, and (*4*) the differences in temperature range, and in optimum, maximum and minimum temperatures of the various organisms for rate of decay during a given time. The presence of *Oospora citri-aurantii* in certain mixtures, especially with *Penicillium digitatum,* caused marked increase in some cases, the rate of decay being more than the sum of the rates due to the two organisms when used separately. The mixture of the two *Pencilliums* showed a depressing effect at most temperatures, except the highest and lowest temperatures used. The presence of *Botrytis cinerea* in certain mixtures also had a depressing effect on rapidity of decay as compared to that of the most rapidly advancing organism of the mixture. Some of the mixtures produced characteristics of color and consistency of decaying tissue that helped to elucidate effects not previously well understood in the orchards and packing-houses.

The brown blight disease of lettuce.—IVAN C. JAGGER.

Brown blight is a new disease of lettuce, which has caused increasing losses in the Imperial Valley of California. It appeared soon after the beginning of lettuce culture

there about 12 years ago. More recently it has been found in Arizona and in several other lettuce sections of California. Plants are attacked in all stages of growth from three or four leaf seedlings to maturity. Young plants become yellowish, more or less mottled, much stunted, and gradually die, while on plants that are not attacked until they have attained some size there are, in addition, conspicuous dead brown streaks and blotches on the leaves. The disease is soil borne. It may occur in the first crop of lettuce to a limited extent and increases rapidly from year to year with constant cropping. No appreciable control has been obtained by growing non-susceptible crops on diseased soil for three or four years. The cause has not been definitely determined but a parasitic organism in the roots is indicated. The fungus *Asterocystis radicis* de Wildeman has been found rather constantly associated, but definite proof of causal or secondary relation is as yet lacking. Highly resistant strains of the New York variety ("Iceberg lettuce") have been developed and are being extensively grown in Imperial Valley. At present "Imperial No. 2" strain is the most extensively used with a considerable acreage of "Imperial No. 3" and a few trial fields of the more recently developed "Imperial No. 6."

The occurrence of Peronospora sparsa Berk. on hot-house roses in southern California.—O. A. PLUNKETT.

Altho *Peronospora sparsa* seems to be common and frequently destructive in Europe, it has received little attention in the United States. It appeared in several greenhouses near the coast of southern California in the early fall of 1927 with the advent of cool foggy nights and warm days. The fungus causes a spotting of young foliage followed by a dropping of the leaflets. Under humid conditions abundant conidiophores and conidia are produced in the spots on the undersides of the leaves. The disease is not serious enough to kill the plants but delays the time of blooming two or three weeks, which keeps the roses off the Christmas market, thus causing considerable loss. The disease seems to be associated with poor air drainage, an excess of humidity, a high temperature during the day and a low temperature at night. It has been controlled successfully by proper ventilation, regulation of the humidity and temperature, and by thorough and frequent spraying with bordeaux 5–5–50.

The development of tomato yellows under different light conditions.—MICHAEL SHAPOVALOV and F. S. BEECHER.

Experiments with artificial shading of tomato plants, which tended to lower the rate of evaporation, reduced the amount of yellows (formerly known as western yellow blight). While some of this reduction under field conditions may have been due to the protection of the plants from beet leafhoppers, instrumental in spreading the infection, it was also attained in cases when tomatoes were artificially inoculated and then shaded. Inoculation tests conducted simultaneously under different habitats show very definitely that light is of primary importance in the progress of tomato yellows and correlates very closely with the rate of evaporation. However, indications are that low relative humidity also favors the disease to a certain extent. The total intensity of light during these experiments was measured at various times by the uranyl acetate-oxalic acid method. The effect of reduced light was four-fold: (*1*) the incubation period in the host was prolonged; (*2*) the plant growth was accelerated; (*3*) the disease symptoms took on a milder form; and (*4*) the percentage of yellows was lower.

The influence of sodium chloride in alkali soils on the susceptibility of Pima cotton to angular leaf-spot caused by Phytomonas malvacera.—J. G. BROWN.

Experiments carried on by the writer since 1921 in the field, screened garden, and laboratory show that Pima cotton grown on soil containing 0.2 to 0.4 per cent of sodium chloride is resistant to angular leaf-spot. When the concentration of sodium chloride is near the limit of tolerance of cotton (0.4 per cent), Pima cotton is practically immune to the disease. Sodium carbonate in the soil shows no protective effect. The resistance observed appears to bear no relation to osmotic concentration. It is probably due to the accumulation of Cl in the parenchyma where it can be detected by microchemical methods. The series of experiments is of interest in connection with the growing of cotton on alkali soils.

Further studies on attenuation of the virus of sugar beet curly-top.—EUBANKS CARSNER and C. F. LACKEY.

In connection with the study of resistance to the curly-top disease, resistant sugar beets have been infected by means of viruliferous leafhoppers and the virus then transferred from these diseased plants to healthy susceptible sugar beets by the use of non-viruliferous leafhoppers. The tests reveal that under certain conditions, which are as yet not well defined, the passage of the curly-top virus through resistant beets results in a marked attenuation of the virus. The degree of attenuation varies to such an extent that when small plants of a susceptible strain are inoculated with the attenuated virus the resulting symptoms range from the severity characteristic of the disease when due to the active or unattenuated virus to the very mild form produced by the virus after it has been passed through *Chenopodium murale*. The variation in degree of attenuation appears to correspond directly to the severity of the symptoms on the infected resistant plant used as the source of the attenuated virus. Attenuation of the virus by resistant sugar beets has been demonstrated for three different strains. The evidence thus far available indicates that neither prolonged development of the attenuated virus in a susceptible plant nor repeated passage of it throught susceptible plants restores its virulence.

Measurements of total daily sunlight intensity with reference to the ecology of plant diseases.—F. S. BEECHER.

Need for a readily portable, inexpensive, and reasonably reliable method for measuring the cumulative effect of sunlight in the study of plant diseases led to the adoption of Dr. R. F. Bacon's chemical photometer method, using uranyl acetate and oxalic acid.

By this method the percentage of total sunlight available to plants under various conditions appeared to be, on the average, about as follows: Open frame 87 per cent, frame with thin single glass 70 per cent, with double glass 60 per cent, thin muslin 47 per cent, medium weight muslin 40 per cent, heavy muslin 8 per cent, shady portion of greenhouse 15 to 20 per cent, central greenhouse 30 to 35 per cent, and sunny portion 35 to 45 per cent. The very small effect under both the Uviarc quartz lamp and the 500-watt Mazda showed how inadequate they are for plant illumination, as compared with sunlight, as well as possibly suggesting that the photochemical effect may be produced by a fairly wide range of the spectrum, rather than being confined to the violet and ultra-violet portion.

As a method for use in the field and greenhouse this chemical photometer has seemed quite satisfactory.

A study of citrus blast and some allied organisms.—C. O. SMITH.

Study has shown a great similarity in the pathogenicity and cultural characteristics of *Bacterium citriputeale* from avocado and Citrus, *Bact. syringae* from lilac in California, and *Bact. cerasus* from apricot. The cultural characteristics are in accord with the description[1] of the lilac organism. The following are some of the hosts that were successfully inoculated by puncture with each of the species mentioned: fruit of *Prunus armeniaca;* twigs of *P. armeniaca* but slightly infected except with *Bact. cerasus* where a lesion about 20 millimeters resulted; fruit and twigs of avocado; twigs of *Chalcas exotica,* one of the Citrus relatives; twigs and leaves of *Coprosma baueri* (a susceptible host); twigs of *Jasminum primulinum, Fraxinus floribunda* (S. P. I. 47687); fruit of lemons; orange twigs, lesions small, but typical; fruit of tomato; lilac leaves and twigs; leaves and twigs of *Populus sp;* fruit and twigs of pears; fruit and twigs of apple. Reisolations and reinoculations were made to complete the rule of proof. From the study thus far given the results seem to indicate that the three species are closely related culturally and pathogenically. They may be found to belong to the same species, but more study is necessary to determine this, and no change is suggested in the nomenclature at present.

Steam sterilization of coniferous seed-beds.—T. C. SCHEFFER.

Experimental work done during the past season to determine the efficacy of steam sterilization by the inverted pan method when applied to coniferous seed-bed soil has indicated excellent possibilities for its use. Three western genera of conifers, Douglas Fir, Sitka Spruce and Western Yellow Pine, were used in the investigation. In the case of each genus, seedling counts made in the fall showed the plants in the control beds to be outnumbered three to four by the plants in the beds which had been steamed in the spring. Root systems of plants in the steamed soil also showed a greater development. Weeding costs for the steamed beds were reduced to but one-sixth of those of the control beds. One-hour and two-hour steaming periods were used, employing low pressure steam, with results indicating that these were in excess of the time actually needed to sterilize sufficiently for fungi. The damping-off organisms involved seemed to be *Fusarium sp.* and *Phoma sp.*

Eocronartium musicola fr. in a new locality and on a new host.—KEITH O'LEARY.

In July of 1926 *Eocronartium musicola* was found on the moss *Leucolepis acanthoneura* (Schwaegr.) Lindb. at Sedro-Wooley, Washington. *Leucolepis* adds a third family, Mniaceae, to the list of hosts, the other families being Leskaceae and Hypnaceae. This is apparently the first time that this interesting fungus has been found in the west. Several attempts were made to culture the fungus on the ordinary laboratory media but all failed. Some field inoculations were attempted by dusting the sporophores over the moss, but this work, too, gave negative results.

Studies of Texas root rot in Arizona.—R. B. STREETS.

Texas root rot caused by *Phymatotrichum (Ozonium) omnivorum* causes the greatest economic loss of any plant disease occurring in Arizona. The life history of the fungus and control measures have been studied the last two years. Efforts to germinate the conidia which are often produced in great abundance have been unsuccessful, although a

[1] BRYAN, MARY K. Lilac blight in the United States. Jour. Agr. Res. 36: 225–235. 1928.

large number of media have been tried. The fungus is polymorphic, advancing through the soil by means of rhizomorphs composed of a large central hypha surrounded by a sheath of finer mycelium bearing the characteristic slender cruciform-branched appendages. The primary infection on cotton and alfalfa is usually on the taproot from 6 to 12 inches below the soil surface. Primary invasion is accomplished by a combined wedging action of the hyphae between the cortical cells and the penetration and filling of the adjacent cells with hyphae. A small recently formed lesion shows scores of points where the fungus has penetrated the cortex. Special technique is necessary to isolate the organism and inoculate healthy plants. One hundred selections from very severely infested fields of Pima cotton and a like number of Acala cotton have been grown for two years on heavily infested soil. Certain of the surviving progenies show promise of a satisfactory degree of resistance. Soil sterilization with chemicals has proved effective when the infected spot is small and cost of treatment is not the limiting factor. Formaldehyde, organic mercury salts, carbon bisulphide, and sulphuric acid were the most effective chemicals used.

Inhibition of enzymatic action as a possible factor in the resistance of plants to disease.—L. J. KLOTZ.

During the course of an investigation which seeks to throw some light on possible bases for the resistance of sour orange (*Citrus aurantium* L.) and for the susceptibility of lemon (*Citrus limonia* Osbeck) to the bark diseases gummosis and decorticosis, caused by *Pythiacystis*, it has been found that the trunk bark of sour orange has a much greater inhibitory or paralyzing influence on the action of certain enzymes found in the dried mycelial powder of the casual fungi than does the trunk bark of lemon. This suggests the possibility that resistance to the invasion of the pathogens may be due to the inhibition of one or more of the enzymes of the fungi by some cellular product of the host, and that a sufficient decrease in this paralyzing power might permit the hyphae to progress rapidly, as they do in the bark of the susceptible lemon, and successfully paralyze the host.

Similar tests with other varieties of Citrus have given results approximating the degree of susceptibility and resistance to Pythiacystis gummosis as indicated by lesions resulting from pure culture inoculations under field conditions.

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